A.M.A.

Archives OF PATHOLOGY

NOVEMBER 1956

VOLUME 62 NUMBER 5

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AMERICAN MEDICAL ASSOCIATION Publication

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VOLUME 62 NOVEMBER 1956 NUMBER 5

ORIGINAL ARTICLES

REGULAR DEPARTMENTS

A. M. A.
ARCHIVES OF PATHOLOGY

Also the Official Organ of the American Society for Experimental Pathology

VOLUME 62 — NOVEMBER 1956 — NUMBER 5

Published monthly by the AMERICAN MEDICAL ASSOCIATION. Editorial and Circulation Offices: 535 North Dearborn Street, Chicago 10, Illinois. Publication Office: Thompson Lane, Box 539, Nashville 1, Tennessee. Change of Address: Notice to the circulation office should state whether or not change is permanent and should include the old address. Six weeks' notice is required to effect a change of address. Second-class mail privileges authorized at Nashville, Tenn., Aug. 6, 1956.

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Nutritional Neuropathy

Chronic Thiamine Deficiency in the Rat

J. D. K. NORTH, M.D., D.Phil., M.R.C.P.
and
H. M. SINCLAIR, D.M., M.R.C.P., Oxford, England

It is generally assumed that a deficiency of thiamine in animals will cause lesions in the peripheral nerves which can be demonstrated histologically.[1] Indeed, the name aneurin for the active principle of vitamin B_1 (thiamine), which was originally proposed by Jansen,[2] was derived by contracting *antineuritic* vitamin. Some have criticized this assumption: Meiklejohn[3] stated that "thiamin has never deserved the title of 'antineuritic vitamin' "; Wintrobe and associates[4] have expressed similar views.

An experimental deficiency of thiamine can be held responsible for changes observed in the peripheral nerves only if (1) the other known vitamins which are required by the rat are supplied, preferably in a crystalline form[4]; (2) the secondary effects of inanition are excluded by the use of pair-fed control animals, and (3) the histological changes do not depend on the Marchi stain alone, since this method is capricious and liable to serious errors in interpretation.* In addition to the above requirements, the experiment must be of sufficient duration to allow structural changes to develop in the peripheral nerves.[8]

Swank[9] and Shaw and Phillips[10] have proved that chronic thiamine deficiency causes degenerative changes in the peripheral nerves of the pigeon. In contrast, Follis[11] and Wintrobe[12] and their associates found no changes in the nervous system of the pig in such a deficiency. The effect of chronic thiamine deficiency on the structure of the peripheral nerves of the rat remains in doubt; Follis,[13] in his monograph, stated: "There is no good evidence that thiamine deficiency leads to structural or functional lesions of the peripheral nerves of the Mammalia thus far studied."

There have been 15 studies of the nervous system of the rat in this deficiency. Some of these investigations involved a deficiency of the whole vitamin B complex;† in others the effects of inanition were not controlled.‡ Inanition-control animals showed lesions in the peripheral nerves which were as advanced as those seen in the deficient rats in three further studies.§ Two of the three remaining experiments‖ relied entirely on the appearance of the nerves under polarized light; this method of examination is open to grave errors in interpretation, and one attempt was made in these two investigations

Submitted for publication June 20, 1956.

From the Laboratory of Human Nutrition, University of Oxford. Formerly Walter Dixon Memorial Scholar, British Medical Association (Dr. North).

* References 5-7.

† References 14-17.

‡ References 18-21.

§ References 7, 22, 23.

‖ References 24-26.

to confirm the findings by other histological techniques. Mannell and Rossiter[27] found that neither in acute thiamine deficiency nor in a deficiency of total calories was there a degeneration of the myelin sheath in a chemical sense. Rodger,[28] who was concerned primarily with the visual pathways of the rat, reported that degenerative lesions were found in the sciatic nerves of rats during chronic thiamine deficiency and that similar changes were not seen in pair-fed control animals.

Experimental Methods

Animals.—Seventy-five adult albino rats were used which were 6 months old at the start of the deficiency. The animals were divided into three groups, as shown in Table 1. Groups A and B each contained 35 rats, which were arranged by sex and weight into 7 pairs and 7 lots of 3 each. Each pair or group of three contained a deficient and a pair-fed control rat. The third animal, which was included in only half of the lots, was a weight-control rat. The weight-control animals were used in case those which were pair-fed failed to control the effects of inanition adequately. Each weight-control rat was pair-fed with its deficient partner unless this rat lost weight more rapidly, in which case the food of the weight-control animal was reduced until its weight loss was the same as that of the deficient animal. The five rats in Group C were healthy animals which were used for the study of the effects of inanition in the other control animals. All the rats were kept in individual cages with wire-mesh floors in a room maintained at a temperature of 72 F.

Diet and Vitamins.—The 70 rats in Groups A and B were fed a purified diet of the following composition: sucrose, 65 parts; casein (fat- and vitamin-free), 20 parts; peanut (arachis) oil, 10 parts; salt mixture, 5 parts ("Salts 4" of Hegsted and associates[29]). Crystalline vitamin supplements were given. The water-soluble vitamins were given orally in a 10% sucrose solution on alternate days. The vitamin mixture was made up so that each rat received the following quantities of pure vitamins daily: riboflavin, 150γ; nicotinic acid, 1 mg.; pyridoxine hydrochloride, 100γ; calcium pantothenate U. S. P., 200γ; para-aminobenzoic acid, 1 mg.; inositol, 2 mg.; choline chloride, 10 mg.; folic acid, 20γ; biotin, 2γ; vitamin K, 100γ. Thiamine was added to this mixture, as indicated in Table 1. Rats that were given the purified diet also received each week adequate amounts of the oil-soluble vitamins oleovitamin A acetate, 1000 I. U.; calciferol U. S. P. (vitamin D_3), 50 I. U.; α-tocopherol N. F., 5 mg.; these were dissolved in 4 drops of peanut oil and given by dropper.

The five rats in Group C were given a diet that contained adequate quantities of all the known nutrients: whole ground wheat, 50 parts; whole ground barley, 25 parts; fish meal, 7 parts; meat and bone meal, 6 parts; dried grass meal,

TABLE 1.—*Arrangement of Rats in Chronic Thiamine Deficiency Experiment*

Group	Division of Rats	Daily Thiamine Intake, γ	Rats Used in Experiment		
			No.	Sex	Age at Start, Mo.
A	Deficient rats	3	14	M & F	6
	Pair-fed control rats	100	14	M & F	6
	Weight-control rats*	100	7	M & F	6
B	Deficient rats	5 (1-100 days) 1.5 (101-193 days)	14	M & F	6
	Pair-fed control rats	100	14	M & F	6
	Weight-control rats*	100	7	M & F	6
C	Stock-diet control rats	Unknown but adequate	5	M & F	6
	Total no. of rats 75				

* The weight-control rats were, at the start of the experiment, pair-fed with the first seven deficient rats in each group.

TABLE 2.—*Method of Histological Examination of the Nervous System*

Part of Nervous System	Side	Part of Sciatic and Posterior Tibial Nerve*	Fixative	Embedding Material	Stain
Sciatic and posterior tibial nerves	Right	b	Osmium tetroxide	Paraffin	Osmium tetroxide
		c	Formalin	Paraffin	Silver
		d	Osmium tetroxide	Paraffin	Osmium tetroxide
		e	Formalin	Paraffin	Silver
	Left	b	Formalin	Paraffin	Modified Marchi
		c	Formalin	Gelatin (frozen sections)	Polarized light and Sudan black
		d	Formalin	Paraffin	Modified Marchi
		e	Formalin	Gelatin (frozen sections)	Polarized light and Sudan black
Peroneal nerve	Right	--	Osmium tetroxide	Paraffin	Osmium tetroxide
	Left	--	Osmium tetroxide	Paraffin	Osmium tetroxide
Lumbar dorsal-root ganglia	--	--	Formalin	Paraffin	Methylene blue
Lumbar cord	--	--	Formalin	Paraffin	Methylene blue

* See Figure 1 for position of each part on the sciatic and posterior tibial nerves.

5 parts; dried brewer's yeast, 5 parts; cod liver oil, 1 part; sodium chloride, 1 part.

The rats were weighed and examined at weekly intervals, when their response to superficial pain stimuli and their ability to walk along a narrow L-shaped runway were noted.

The deficient, pair-fed control, and weight-control rats in each subgroup were killed when the deficient rat became moribund. The animals were killed by bleeding from the carotid artery after being lightly anesthetized with pentobarbital (Nembutal) sodium.

Histological Methods.—Portions of the nervous system were fixed, embedded, and stained, as indicated in Table 2. The sciatic and posterior tibial nerves were divided arbitrarily into five equal parts (Fig. 1), and the pieces were cut in longitudinal section. Most nerve tissue was exposed and fixed in situ in formol-saline for 24 hours before the dissection was completed; this prevented damage and excess shrinkage of the tissue. Pieces of the right sciatic nerve, however, were attached in situ to small glass rods to prevent distortion during fixation in osmium tetroxide. The chlorate-osmium-tetroxide modification of the Marchi stain was employed.¶

Pieces of the peroneal nerves, removed from the lateral side of each popliteal space, were cut in transverse sections for myelinated nerve fiber counts. A section of each nerve was photographed and enlarged 300 times, so that even the smallest myelinated nerve fibers were clearly visible on the print, and these were counted by a counter which recorded automatically each time the photomicrograph was perforated.

¶ References 29-31.

Results

Time of Survival and Changes in Weight.—Individual rats showed a wide variation in their ability to survive during chronic thiamine deficiency. Details of the times of survival and changes in weight are given in Table 3. Because of the small losses of weight during the first 100 days among the deficient rats in Group B (given 5γ of thiamine hydrochloride daily), these rats were then subdivided. Five of the animals which had lost most weight were killed, although not moribund from the deficiency (Group B.1), and the remaining nine rats (Group B.2) had their daily thiamine intake reduced to 1.5γ. One deficient rat which had lost 75 gm. when fed 3γ of thiamine hydrochloride for 200 days showed a dramatic increase in weight (26 gm. in 48 hours)

TABLE 3.—*Times of Survival and Losses of Weight of Rats During Chronic Thiamine Deficiency*

Group	Type of Rat	Daily Thiamine Intake, γ	No. of Rats	Average Survival Time, Days	Longest Survival Time, Days	Average Initial Weight, Gm.	Average Weight at Death, Gm.	Average Loss of Weight, Gm.
A	Deficient	3	13*	165	255	263	179	84
B.1	Deficient	5	5	103 (killed)	106 (killed)	278	243	35
B.2	Deficient	5 for 100 days, then 1.5	9	136	193	234	155	79
C	Stock-diet control	Adequate	5	230 (killed)	255 (killed)	246	288	+ 42 (gain)

* One rat was excluded, as it was treated with 100 γ of thiamine chloride daily from the 200th day.

when the thiamine intake was increased to 100γ daily.

"Clinical" Examinations.—Convulsive seizures were rarely seen among the deficient rats, and only one rat, fed 1.5γ of thiamine hydrochloride daily, developed spontaneous seizures. No control rats suffered from convulsions. The test for ataxia revealed only slight differences between the deficient and the inanition-control animals. All rats which lost weight were prone to make mistakes on the runway; this was an effect of inanition. One deficient rat, however, showed definite ataxia, although the animal was quite strong at the time the disorder developed, 12 days before its death. The animals which had been on a deficient diet did not show any alteration in their response to pain stimuli.

Rats Employed in the Histological Study.—The experiment involved the use of adult rats and lasted over 36 weeks; as a result some animals showed, at postmortem examination, evidence of pathological changes

TABLE 4.—*Histological Examination of the Nervous System in Chronic Thiamine Deficiency*

Group	Type of Rat	Daily Thiamine Intake, γ	Rats Examined Histologically	Average Survival Time, Days	Rats Showing Degenerative Lesions in Nervous System					
					Sciatic and Posterior Tibial Nerve				Lumbar Dorsal-Root Ganglia	Lumbar Cord
					Right		Left			
					Silver Stain	Osmium Tetroxide Stain	Sudan Black Stain	Modified Marchi Stain		
A	Deficient	3	6	181	1	2	2	3	--	--
	Inanition-control	100	6	181	--	--	--	1	--	--
B.1	Deficient	5	4	103	--	--	--	--	--	--
	Inanition-control	100	4	103	--	--	--	1	--	--
B.2	Deficient	5 for 100 days; then 1.5	5	157	3	4	4	4	--	--
	Inanition-control	100	5	157	--	--	--	--	--	--
C	Stock-diet control	Adequate	5	230	--	--	--	--	--	--

unrelated to the deficiency. Pneumonic consolidation, to which this strain of albino rats was prone, was the commonest lesion, and animals suffering from this disorder were excluded. Rats which died during the night were also omitted because a preliminary experiment (unpublished) showed that postmortem autolytic changes occurred in both the cell bodies and the peripheral nerves 15 hours after death. All the remaining rats were studied histologically, but in those groups in which both types of inanition-control rats were available only the weight-control rats, which suffered more severely from starvation, were examined. The results of the histological investigation are given in Table 4.

Myelin Sheaths in the Sciatic and Posterior Tibial Nerve.—The myelin was stained with osmium tetroxide in two segments of the right nerve; it was stained with Sudan black and examined under polarized light in two parts of the left nerve. Inanition alone did not cause widespread degenerative changes in the myelin. The number of fine droplets of myelin in the individual nerve fibers (observed with the osmium tetroxide stain) was reduced after inanition. No similar changes were noted with Sudan black, as this substance stained the neurokeratin more deeply than the myelin droplets.[32]

Sections from a minority of the nerves of inanition-control rats showed an isolated fiber that was definitely degenerating. Not more than two such fibers were seen in one section of any nerve. As the rats were over a year old when they were killed, age alone could have been responsible for an occasional degenerating fiber,[39] and starvation probably enhanced this tendency. The rare degenerating fibers occurred as frequently in the proximal as in the distal segment of the nerve; this suggests that the cell bodies of these fibers were dying. If the degenerating fibers had been the result of "toxic" influences on healthy neurons, the degeneration would almost certainly have been observed more commonly in the more distal segment of the nerve.

In the deficient rats similar isolated degenerating fibers were excluded in searching for evidence of degeneration. Definite degenerative changes, however, were seen in the myelin sheaths of the posterior tibial nerve in some of the deficient animals, particularly in those which underwent the more acute deficiency of thiamine (Table 4). Degenerative changes were observed only in the distal parts (segments *d* and *e*, Fig. 1) of the sciatic and posterior tibial nerves. When degeneration occurred, there were between 10 and 40 degenerating myelin sheaths in a single section (Fig. 2); the changes were similar in character to those seen in the distal stump of the nerve after surgical division.

Examination under polarized light revealed degenerative changes in most of the nerves that were shown to be degenerating by the Sudan black stain, but these changes were more difficult to detect; they included both "sausage-link" structures and accumulations of bright anisotropic globules, frequently in the form of "Maltese crosses."

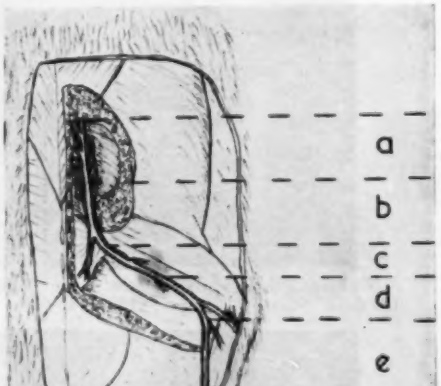


Fig. 1.—Schematic diagram of the sciatic and posterior tibial nerve of the rat, exposed from the dorsal aspect to show the arbitrary method of division of the nerve.

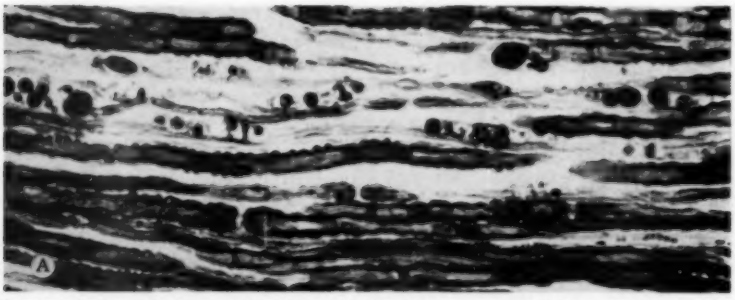
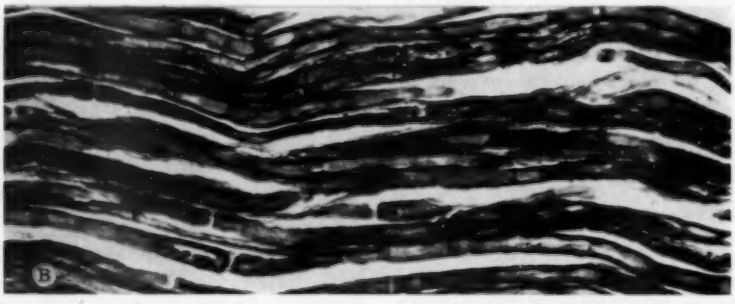
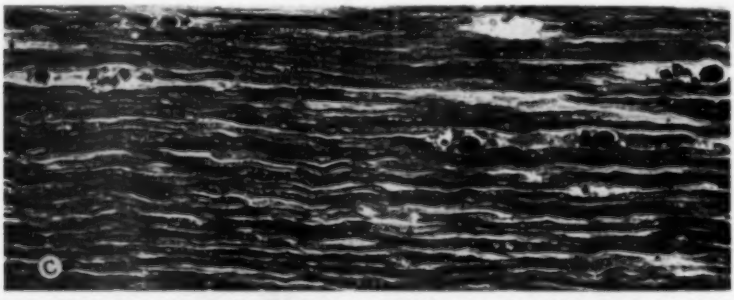


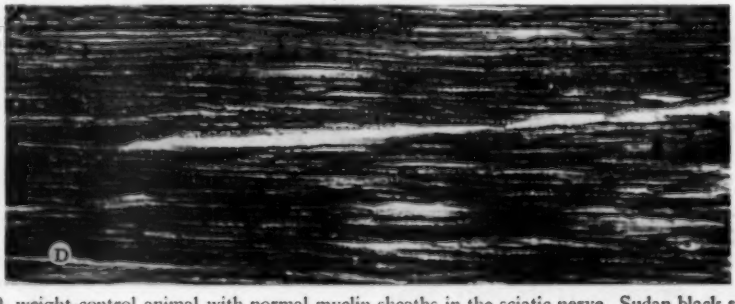
Fig. 2.—*A*, degenerating myelin sheaths in the distal segment of the sciatic nerve of a deficient rat which survived 161 days. Osmium tetroxide stain; reduced to 60% of mag. × 600.



B, more proximal segment of the same nerve as that shown in *A*, with intact myelin sheaths. Osmium tetroxide stain; reduced to 60% of mag. × 600.



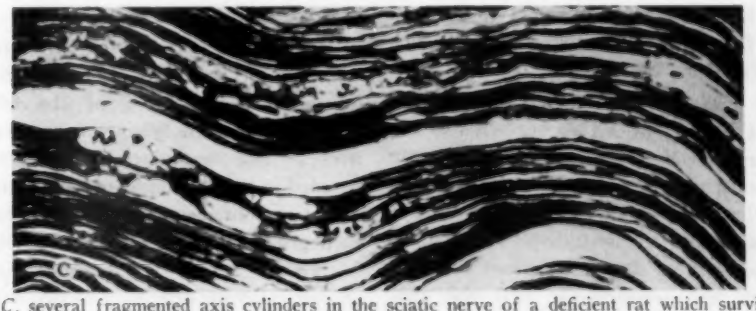
C, several degenerating myelin sheaths in the sciatic nerve of a deficient rat which survived 189 days. Sudan black stain; reduced to 60% of mag. × 400.



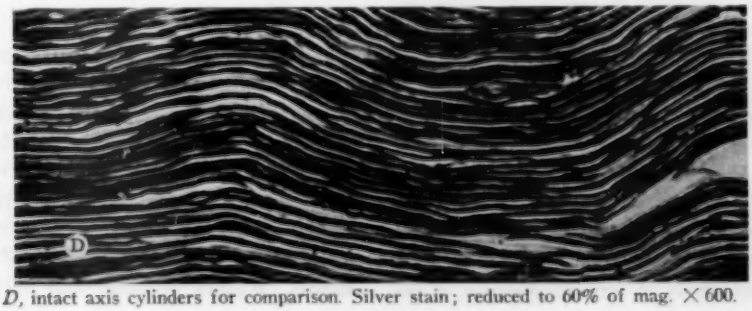
D, weight-control animal with normal myelin sheaths in the sciatic nerve. Sudan black stain; reduced to 60% of mag. × 400.

Fig. 3.—*A*, sciatic nerve of a deficient rat which survived 206 days. There are numerous fragmented myelin sheaths. Modified Marchi stain; reduced to 60% of mag. × 400.

B, stained but intact myelin sheaths which were seen in most of the inanition-control animals. The "dust" of osmium tetroxide can be seen in the background. Modified Marchi stain; reduced to 60% of mag. × 400.



C, several fragmented axis cylinders in the sciatic nerve of a deficient rat which survived 206 days. Silver stain; reduced to 60% of mag. × 600.



D, intact axis cylinders for comparison. Silver stain; reduced to 60% of mag. × 600.

Marchi Stain for Degenerating Myelin.—The modified Marchi method was used for staining two segments of the left sciatic nerve. The nerve fibers of the stock-diet-control rats remained unstained, but a majority of the nerves of both the inanition-control and the deficient animals had many fibers which, although normal in appearance, were stained black by the Marchi solution (cf. Swank[9]). This change was a result of inanition. If only fragmented fibers and globules of myelin were noted, the modified Marchi method was of limited value. The globules of degenerating myelin were readily distinguished from nonspecific deposits of osmium tetroxide called "dust," as the globules tended to occur in groups and were larger in size than the particles of "dust." All rats in which degenerative changes were found in the sciatic and posterior tibial nerve with normal myelin stains showed degenerating fibers (Fig. 3). However, one extra deficient animal and two inanition-control rats also had nerves in which some fibers appeared to be degenerating. In these three rats the Sudan black stain of the more distal segment of the same nerve failed to verify the changes, which were therefore regarded as artifacts.

Axis Cylinders in the Sciatic and Posterior Tibial Nerve.—Two segments of the right nerve were stained with silver nitrate to demonstrate the axis cylinders. Normal axis cylinders in both deficient and control animals showed a wide individual variation. In some sections the shrinkage of the axis cylinders was uniform, so that the axis cylinders appeared as fine black threads of constant diameter; in others the shrinkage was irregular, and consequently the apparent diameter of the axis varied. An occasional normal axis cylinder, instead of being a compact unit, appeared to form a latticework. Constructions at the nodes of Ranvier, too, varied in degree from nerve to nerve. These appearances occurred with equal frequency in the deficient, the inanition-control, and the stock-diet rats and were not regarded as of any significance in detecting degenerative changes in the axis cylinders. Fragmentation and disintegration of the axis cylinder were the only criteria accepted as evidence of degeneration. In some inanition-control rats an isolated degenerating axis cylinder was detected. This corresponded to the rare degenerating myelin sheaths seen after staining with osmium tetroxide and Sudan black; two such axis cylinders were the maximum detected in any one section. In the deficient rats definite changes were seen, but only in rats in which the myelin stains also showed degenerative changes. In all but two of these deficient rats the changes in the axis cylinders were definite (Fig. 3).

Lumbar Dorsal-Root Ganglia and Lumbar Cords.—Two ganglia were examined from each rat. There was some variation in the incidence of chromophilic neurons, but in no ganglion did these neurons exceed 10%. The dorsal-root ganglia and the lumbar cord of the deficient rats appeared similar to those of the inanition-control rats; there were no signs of degeneration comparable to those seen in the distal segments of the sciatic and posterior tibial nerves.

Myelinated Nerve Fiber Counts.—The nerve fiber counts were undertaken to obtain an objective indication of the effect of chronic thiamine deficiency on the peripheral nerves. The myelinated nerve fibers were counted in both peroneal nerves of three deficient and three inanition-control rats from each group; the results are given in Table 5. These counts gave no indication that chronic thiamine deficiency caused degeneration of the peripheral nerves.

Comment

The results obtained in this experiment prove that a chronic deficiency of thiamine does cause degenerative changes in the peripheral nerves of the rat which can be demonstrated histologically. The nervous system of this animal, however, is resistant to the deficiency, and degenerative changes were observed in only a proportion of the animals. Rodger[28] found that only some

rats showed degenerative lesions in the visual pathway after chronic thiamine deficiency.

The rats in which the nervous system was examined were maintained for an average of 181 days on 3γ of thiamine hydrochloride daily, or for 157 days first on 5γ and then on 1.5γ of thiamine daily. The period of deficiency, therefore, represented a considerable portion of the total life of the animals. Rodger [28] maintained rats for 180 days on daily doses of either 2γ or 5γ of thiamine hydrochloride, depending on the degree of anorexia, but killed most of the animals before they were moribund. Prickett and associates [26] are the only other workers who have induced a chronic thiamine deficiency. They maintained rats for 3 to 99 days after the onset of signs of an acute deficiency by feeding the rats small amounts of thiamine each day. In their study the peripheral nerves were examined only under polarized light. These workers reported more extensive myelin degeneration in the deficient rats than was observed in pair-fed control rats. The control rats, however, were pair-fed with rats which died of an acute thiamine deficiency. The pair-fed control rats of Rodger [28] failed to control adequately for the effects of inanition; during the deficiency the control rats gained on an average 129 gm. more than the deficient animals. Such a disparity was not observed in the present experiment. The 14 pair-fed control rats in Group A lost on an average only 10 gm. less than their deficient partners because some pair-fed control rats were hungry and excessively active. During total inanition Wald and Jackson [33] and Siegel and Steinberg [34] showed that the activity of the rat increased greatly. Weight-control rats were first used by Deane and Shaw [35] when studying the influence of thiamine deficiency on the adrenal cortex. Follis [13] recommended the use of this type of control animal, but few workers have employed

TABLE 5.—*Myelinated Nerve-Fiber Counts of the Left and Right Peroneal Nerves*

Group	Type of Rats	Daily Thiamine Intake, γ	Individual Nerve Fiber Counts	Mean of Counts	Standard Deviation
A	Deficient	3	1602 1648 1775 1777 1796 1824	1737	89.7
	Pair-fed and weight-control	100	1681 1691 1696 1703 1712 1818	1717	80.7
B.2	Deficient	5 γ for 100 days 1.5 γ from 101 days	1429 1501 1720 1733 1737 1744	1644	140.7
	Pair-fed and weight-control	100	1455 1594 1667 1675 1734 1860	1666	138.3
C	Stock-diet control	Adequate	1581 1631 1637 1638 1659 1664	1635	29.5

them because of the constant attention they require. Owing to the adequate losses of weight of the pair-fed control animals, the inclusion of weight-control rats was an additional precaution rather than an essential measure.

Under conditions of chronic thiamine deficiency only a proportion of the animals were affected. The changes, when they occurred, were observed in the nerves of the same animals by the osmium tetroxide stain, Sudan black stain, polarized light examination, Marchi stain, and the silver stain for axis cylinders. The results of each method were reported separately and correlated only at the end; and conformity of results, rather than difference, was the outstanding characteristic. Examination of the myelin sheaths showed that osmium tetroxide and Sudan black were superior both to the modified Marchi method and to polarized light examination. Examination under polarized light was at the best only an ancillary method. The results of this method were temporary, preventing repeated comparisons, and the type of change was open to a considerable error in interpretation. Some fibers, which appeared definitely to be degenerating when the Nicol prisms were uncrossed and the intensity of illumination was reduced, were not observed in polarized light because the myelin had become isotropic and merged with the black background. In addition, changes similar to degeneration could be produced readily by failing to place the crossed Nicol prisms at the point of greatest birefringence. Photomicrographs which have been reproduced in several of the earlier papers are unconvincing. It is well known that errors are likely to occur with the conventional Marchi stain.[5] The present investigation confirmed the vagaries of the chlorate-osmium tetroxide modification of the Marchi method. If the nerves had been studied in transverse section, false conclusions would inevitably have been drawn.

The incidence of degenerating nerve fibers in the affected rats was difficult to assess. On longitudinal section degenerating nerve fibers were obvious, and as many as 40 such fibers were seen in a single section of the sciatic nerve. In the rat with the most advanced degeneration, about 7% to 10% of the total number of nerve fibers were affected. In the majority of rats in which degeneration was observed the incidence of degenerating nerve fibers was only 3% to 5%. This is considerably less than the 10% to 15% of degenerating fibers reported by Vedder and Clark[36] and McCarrison[37] in pigeons during vitamin B complex deficiency. These workers relied on the Marchi stain.

In the rats showing degenerative changes in the sciatic nerves the lesions were restricted to the distal segments *d* and *e*. It was not surprising, therefore, that the neurons in the lumbar dorsal-root ganglia and in the lumbar cord did not show degenerative changes. Immersion fixation, provided it was done soon after death, was quite satisfactory, and the results were much superior to those indicated by Koenig and Koenig[38] for this method.

In inanition the myelin sheaths stained with osmium tetroxide showed a reduction in the number of fine myelin droplets, similar to those first reported by Swank.[9] With the chlorate-osmium tetroxide modification of the Marchi method it was found during inanition that many normal fibers stained black (cf. Swank[9]). Inanition certainly did not cause the "especially extensive disintegration of myelin" when the sections were examined with osmium tetroxide or Sudan black, as was reported by Davison and Stone.[22]

The peroneal nerve fiber counts in stock-diet control rats were similar to those reported by Duncan,[39] although lower than the average value of 2022 reported by Greenman.[40] There was a wide individual variation in the peroneal nerve fiber count; the deficient rats did not show lower average values than the inanition-control animals, thus confirming the suggestion that the nervous system of the rat is relatively resis-

tant to thiamine deficiency. In pigeons during chronic thiamine deficiency, Swank[9] did not compare the deficient birds with pair-fed controls, but in a regeneration experiment after chronic thiamine deficiency he found that the myelinated nerve fiber count of the sciatic nerve rose from 6155, before, to 13,035 after, 90 days of therapy with thiamine. In cases of peripheral neuropathy in man a gross decrease was observed in the number of meylinated nerve fibers in the anterior tibial nerve. The normal value is 6500 to 9000 per square millimeter. In alcoholic peripheral neuropathy a value as low as 1075 per square millimeter was observed by Greenfield and Carmichael,[41] while Aring and associates[42] obtained a count of 380 per square millimeter in one case of "neuritis complicating pellagra." The peroneal nerve count was taken at a level which corresponded to the upper half of segment *d* in the posterior tibial nerve; this was close to the level where degenerative changes ceased even in the affected rats.

When compared with other species, the rat appears to fall halfway between the pigeon and the pig. The nervous system of the pigeon is very susceptible to chronic thiamine deficiency.# When Swank found such convincing histological signs of degeneration in the nervous system, the chronic deficiency of thiamine lasted between 27 and 49 days. In the present experiment only half the rats showed degenerative changes after about 150 days of deficiency. The nervous system of the pig failed to show signs of degeneration in chronic thiamine deficiency after 258 days.[12]

Summary

The effect of chronic thiamine deficiency on the histological structure of the nervous system was investigated in an experiment in which 75 rats were used. Of these, 28 rats either were given 3γ of thiamine hydrochloride daily (average survival time 165 days) or were supplied with 5γ of thiamine hydrochloride daily for 100 days, after which the daily thiamine intake was reduced to 1.5γ (average survival time 156 days). The effects of inanition were controlled by using 28 pair-fed and 14 weight-control rats.

"Clinical" examinations, including a test for ataxia and one for pain sensation, revealed no conclusive exidence of peripheral nerve degeneration.

On histological examination, definite signs of nerve degeneration were seen in the distal segments of the sciatic and posterior tibial nerves. These changes were observed with the Sudan black stain, osmium tetroxide stain, modified Marchi stain, and polarized light examination of the myelin sheaths; similar changes were seen also in the axis cylinders. In most rats which had degenerating nerves about 3% to 5% of the fibers were affected. Only some of the deficient rats, however, showed histological signs of degeneration.

The neurons in the lumbar dorsal-root ganglia and in the lumbar cord showed no evidence of specific degeneration in chronic thiamine deficiency.

In some rats the number of myelinated nerve fibers in the peroneal nerves was counted. The results for the deficient rats were not significantly different from those obtained from the inanition-control animals. Inanition itself caused some changes in the peripheral nerves, particularly blackening of the fibers, with the modified Marchi method.

It is concluded that the nervous system of the rat is relatively resistant to the effects of chronic thiamine deficiency, but that if the deficiency is of sufficient length and intensity degenerative changes do occur in the peripheral nerves.

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Mr. E. H. Leach, of the Laboratory of Physiology, the University of Oxford, prepared the photomicrographs. Roche Products, Ltd., provided the vitamin preparations.

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Transplantable Leydig-Cell Tumors in Mice

Their Physiologic and Pathologic Effects

KELLY H. CLIFTON, Ph.D.
ERIC BLOCH, Ph.D.
ARTHUR C. UPTON, M.D.
and
JACOB FURTH, M.D., Boston

The Leydig-cell tumor herein described had several remarkable effects on its hosts, many of which have not hitherto been ascribed to interstitial-cell tumors. These included masculinization, obesity, adrenocortical atrophy, stimulation of the uterine horn with the formation of deciduoma, pronounced stimulation of erythropoiesis, and, frequently, death from exsanguinating pleuropericardial hemorrhage. Increased quantities of two ketosteroids were found in the urine of tumor-bearing mice.

Historical Review

Interstitial (Leydig)-cell tumors of the testis rarely occur spontaneously [1] but have been induced experimentally in mice by prolonged administration of estrogens. Burrows [2] noted Leydig-cell hyperplasia in mice receiving estrogens. Gardner [3] and Bonser [4] found that the response of the testes to estrogens varied with the strain of mouse. Testicular tumors were common in estrogen-treated mice of Strain A, and some showed histologic evidence of malignancy.[5] One tumor was successfully transplanted to a normal female and to estrogen-treated males.[4] Investigating the histogenesis of the tumors, Hooker and Pfeiffer [6] noted that the male accessory sex organs, which atrophy during the first weeks of estrogen treatment, became enlarged when the tumors grew, indicating androgen secretion. Growth of estrogen-induced Leydig-cell tumors was at first dependent on treatment with estrogens, but after several generations of transplantation, the tumor cells acquired autonomy.* Biskind and Biskind [8] and Twombley and associates [9] induced Leydig-cell tumors by transplantation of infantile testis to the spleens of castrate rats. In these, sex hormones, if secreted, would have been inactivated in the livers of the hosts.

Materials and Methods

Origin of Rf Tumor Strain.—The Leydig-cell tumor studied extensively arose spontaneously in an untreated male mouse of the Rf strain. The rate of occurrence of such tumors in this strain is less than 1%. The mouse was killed when 492 days of age. It was well developed and nourished but not obese. The left testis was normal; the right bore a spherical tumor, which measured about 1 cm. in diameter and caused atrophy of the seminiferous tubules. About half the tumor was necrotic; the rest was yellow and soft and was found to be composed of large, nearly normal-appearing Leydig cells with scanty stroma.

Two other Leydig-cell tumors were studied. One, studied in two transplant generations, arose spontaneously in a mouse of the BALB/c strain and caused uterine hypertrophy and masculinization in female recipients.[10] The other arose in a male of the LAF_1 strain that had been exposed to radiation from an atomic detonation and was studied in two transplant generations.

Recipients.—All Rf animals were raised in our laboratories. BALB/c and LAF_1 mice were ob-

Submitted for publication June 11, 1956.

Fellow in Cancer Research of the American Cancer Society (Dr. Clifton).

This investigation was supported by research grant C-2259 from the National Cancer Institute, U. S. Public Health Service. Work at Oak Ridge was done under USAEC Contract W-7405-Eng-26.

Children's Cancer Research Foundation, Inc., Department of Pathology, Harvard Medical School; the Worcester Foundation for Experimental Biology, Shrewsbury, Mass., and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

* References 6, 7.

tained from the Jackson Memorial Laboratory. The animals were given Purina Chow and tap water ad libitum, supplemented with fresh carrots or lettuce once weekly.

Transplantation.—Tumor tissue was minced and suspended in saline. Approximately 0.05 ml. of the suspension was injected into the muscle of the thigh of isologous mice.

Autopsies and Tissue Studies.—All animals were autopsied. Often, comparative autopsies were performed on tumor-bearing animals killed with normal animals of the same age or with animals that failed to develop tumors after tumor grafts. Tissues were fixed in Zenker-formol solution and stained by standard procedures.

Urine Collections and Steroid Analyses.—Urine samples from two Rf-tumor-bearing mice were collected for five days and stored with 10%, v/v sulfuric acid. After all samples were combined (total volume, 31 ml.), creatinine and total 17-ketosteroid concentrations were determined. Separation and determination of individual 17-ketosteroids were accomplished by a modified Rubin procedure,[12] employing paper chromatography. The urine-pool extract was chromatographed in the solvent system heptane-propylene glycol for 24 hours. The chromatogram was divided into four sections, each section eluted, and the eluates concentrated and rechromatographed in the same system or in heptane-ethylene glycol phenyl ether[12] for 8 to 48 hours. Detection and tentative identification of the 17-ketosteroids present on the chromatogram were achieved by color tests[13]; the reactive zones were eluted and quantitative determinations carried out.

Results

Transplantation.—Thirty-five passages (ten generations) of the Rf tumor were made over the course of four years (Table 1). All passages were successful, but not in all animals. No significant sex preference was noted.

Evidence indicated considerably diminished hormone secretion during the last five generations, and therefore only the first five generations are treated in detail here. These tumors developed in 85 of 132 injected males (64.4%) and in 88 of 143 females (61.5%). There was a slight increase in the percentage of takes with successive generations.

The latency period was long. In the first passage the tumors were palpable after an average of 237 days in the males and 338 days in the females. In subsequent passages latency was reduced to as short a period as 20 days. Growth was similarly slow, several animals living for more than 200 days after the tumor graft was first palpable.

Several transfers of the Rf tumor were made in radiothyroidectomized hosts because radiothyroidectomy of mice leads to the development of thyrotropic pituitary tumors,[13] and grafts of such pituitary tumors in athyroid mice often cause hyperplasia of the Leydig cells.[14] It was, therefore, assumed that radiothyroidectomy might stimulate growth of Leydig-cell tumors. However, the reverse was found. In five experiments 9 of 32 (28%) grafted radiothyroidectomized males and 3 of 18 (17%) females developed tumors.

In general, secondary changes in radiothyroidectomized animals bearing Rf-Leydig-cell tumors resembled those in intact hosts. The following differences were

TABLE 1.—*Transplantation Data of Leydig-Cell Tumors (Strain Rf)*

Passage	Males		Females	
	No. +/No. Inj.	Latency Avg. (Range), Days	No. +/No. Inj.	Latency Avg. (Range), Days
Original	3/8	237 (117-341)	2/7	338 (117-558)
I	15/24	108 (85-210)	1/16	93
II	21/30	99 (35-217)	24/36	65 (43-157)
III	26/47	71 (26-242)	32/43	53 (26-89)
IV	10/14	88 (32-254)	21/27	62 (25-177)
V	11/16	52 (20-111)	18/22	57 (20-126)
VI- X	35/42	35 (10-130)	33/43	28 (10-117)

noted: The mammary glands of the radiothyroidectomized females were markedly hyperplastic. The ovaries of euthyroid tumor-bearing mice were characteristically devoid of lutein cells, whereas those of the radiothyroidectomized mice contained many luteinized stroma cells. The pituitary glands of the I^{131}-treated animals exhibited the usual changes that follow thyroidectomy and terminate in tumor formation.[15]

The low-secreting LA tumor showed sex specificity. In the original passage, tumors developed in 5 of 8 males and in none of 5 sibling females, and in the first-generation subpassage, in 16 of 16 males and in only 3 of 6 females. Latency of grafted tumors was also longer in females (311-364 days) than in males (192-311 days). These observations are remarkable because they indicate that virulence and secretory capacity do not go hand-in-hand and that responsiveness to a secondary factor can exist without distinct evidence of hormonal secretions.

The BALB/c tumor grew equally well in all hosts of both sexes, and there was no indication of sex preference. The latency period in males averaged 37 days, and in females, 44 days.

Morphologic Characteristics of the Tumor Grafts.—The Rf-Leydig-cell tumors were soft and pale yellowish-brown, resembling grafted luteomas † and adrenocortical tumor in color and consistency.[16] The tumors were poorly vascularized and had a marked tendency toward necrosis. Often there was only a narrow rim of "living" tissue about the opaque, yellow-white necrotic mass. Hemorrhages were common but not extensive. Variations in the number of viable cells in the inocula may have, in part, accounted for the great variation in the percentage of takes and the length of latency periods. The microscopic appearance of these tumors was described by Dunham and Stewart.[17] Rf-tumor cells resembled large, active Leydig cells (Fig. 1). Nuclei were relatively uniform in size and occupied about one-third of the total cell volume. The nuclei were round or oval, or less commonly bean-shaped or indented, and the nuclear membrane was thin. Chromatin was usually evenly distributed. The nuclei contained one or two basophilic nucleoli, and occasionally round inclusions. The cytoplasm contained fine acidophilic granules, and often numerous fine vacuoles, probably lipid droplets.

In larger tumors viable cells were found predominantly on the outer rim and in the perivascular regions. Cords or sheets of cells were tranversed by sinusoids. Connective tissue septa, continuous with an imperfect capsule, divided the tumor into nodules. Foci of hemopoietic (mainly erythropoietic) cells were common in healthy tumors. Extravascular degenerating blood pools occurred about necrotic areas, with occasional foci of leucocytes and hemosiderin-containing macrophages.

The LA tumor was similar in gross appearance to the Rf tumor. Tumor cells were of relatively uniform size, without marked anaplastic features, and likewise appeared in cords or sheets (Fig. 3). Thus, lack of secretion was not accompanied by dedifferentiation.

The BALB/c tumor was similar except that the size of nuclei of tumor cells was more variable and the nuclei commonly contained PAS-positive inclusions (Fig. 4). The latter is probably a degenerative change. Mast cells were common, and sometimes numerous, in both the Rf (Fig. 5) and the BALB/c tumors. They were commonest near blood sinuses and septa, but also occurred within the sheets of tumor cells.

It is remarkable that after four years of passages, during which the growth rate of the Rf tumor markedly increased, metastases did not progress beyond the regional lymph nodes (Fig. 2). Even these, though enlarged, were often free of metastases, enlargement resulting from inflammation and hemorrhage.

† Cohen, A. I., and Furth, J.: Histologic and Physiologic Characteristics of Secreting Transplantable Adrenal Tumors in Mice and Rats, in manuscript.

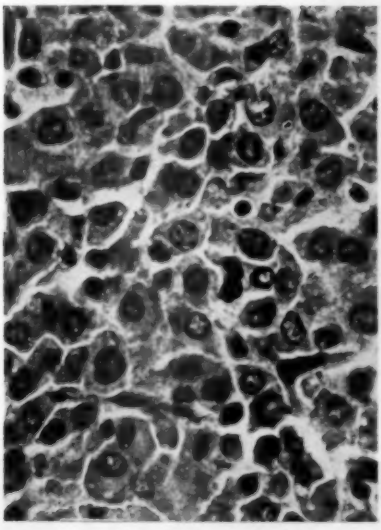


Fig. 1.—Leydig-cell tumor (Strain Rf). Tumor cells are of relatively uniform size; nuclear-cytoplasmic ratio is somewhat reduced. Hematoxylin-eosin; × 630.

Fig. 2.—Metastasis in iliac node of the animal in which the Rf tumor arose. Hematoxylin-eosin; × 110.

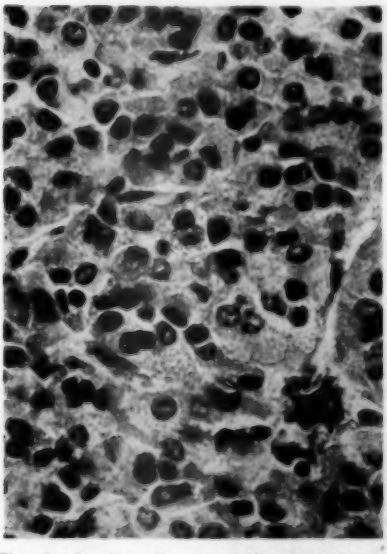


Fig. 3.—Leydig-cell tumor (Strain LA). The tumor cells are smaller than those of the other two strains and are fairly uniform in size and shape. Nuclear-cytoplasmic ratio is reduced. Hematoxylin-eosin; × 630.

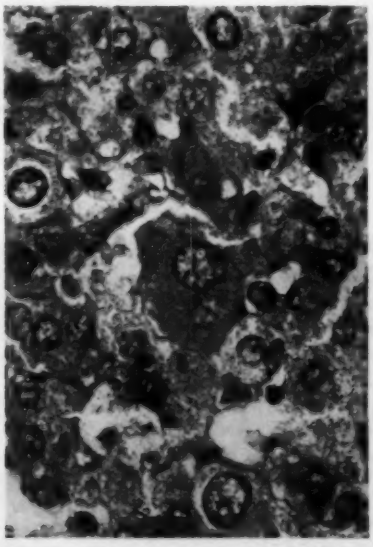


Fig. 4.—Leydig-cell tumor (Strain BALB/c). There is great variation in volume of nuclei and cytoplasm of tumor cells. Giant cells are abundant. Hematoxylin-eosin; × 630.

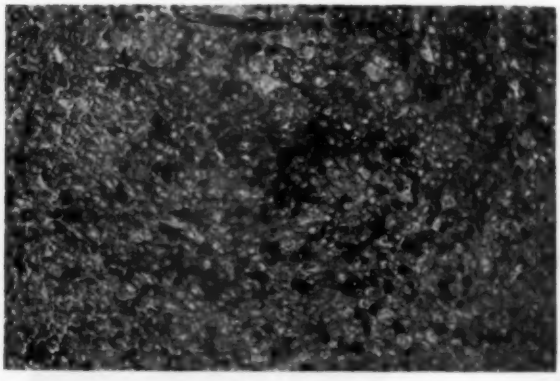


Fig. 5.—Abundant mast cells (dark cells) in Rf-Leydig-cell tumor. Giemsa stain; × 150.

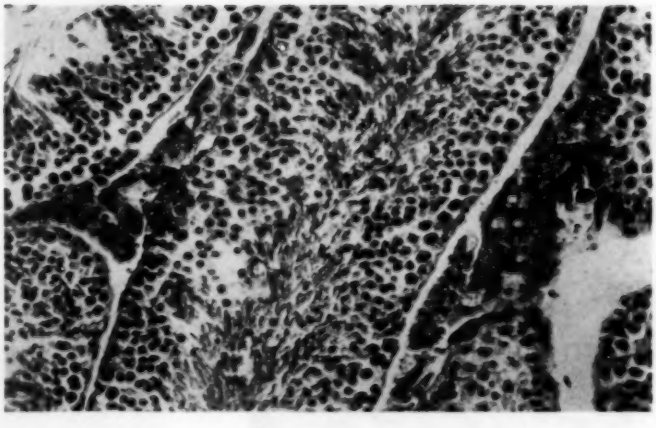


Fig. 6.—Testis of 13-old-month control mouse of the Rf strain, showing normal Leydig cells. Hematoxylin-eosin; reduced to 72% of mag. × 300.

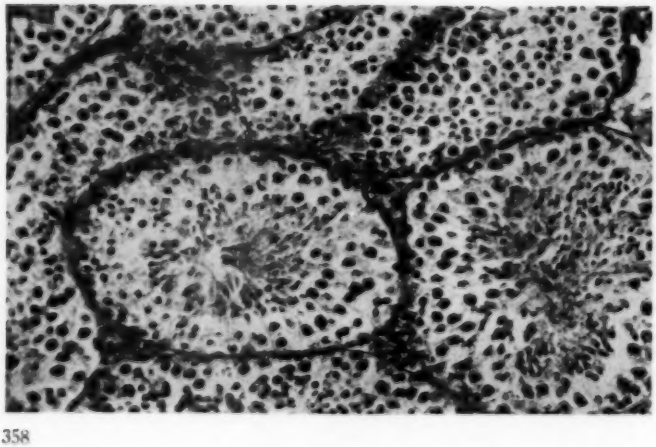


Fig. 7.—Testis of a 13-month-old mouse bearing a large Rf-Leydig-cell tumor, showing absence of Leydig cells. Hematoxylin-eosin; reduced to 72% of mag. × 300.

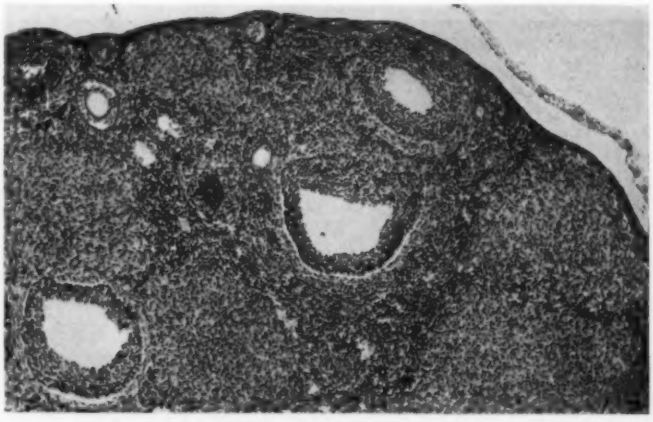


Fig. 8.—Ovary of a normal 6-month-old mouse of the Rf strain killed during the luteal phase of the cycle. Hematoxylin-eosin; reduced to 72% of mag. × 90.

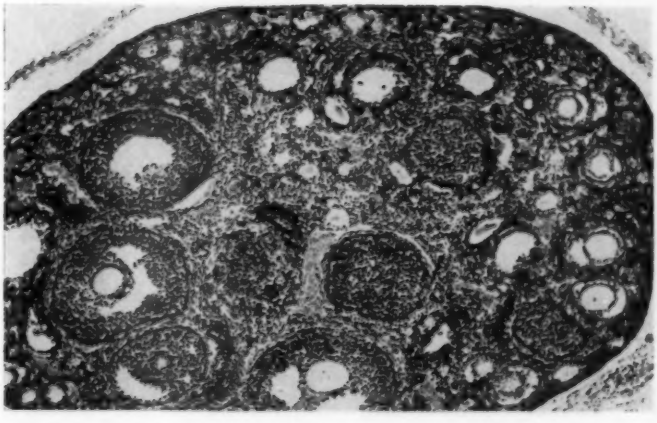


Fig. 9.—Ovary of a 6-month-old mouse bearing a large Rf-Leydig-cell tumor. Note the persistence of follicles and absence of luteinization. Hematoxylin-eosin; reduced to 72% of mag. × 70.

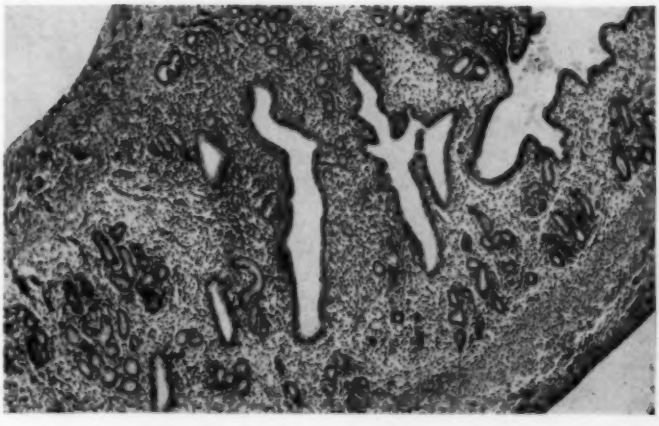


Fig. 10.—Uterus of same normal mouse from which Figure 8 was taken. Hematoxylin-eosin; reduced to 72% of mag. × 90.

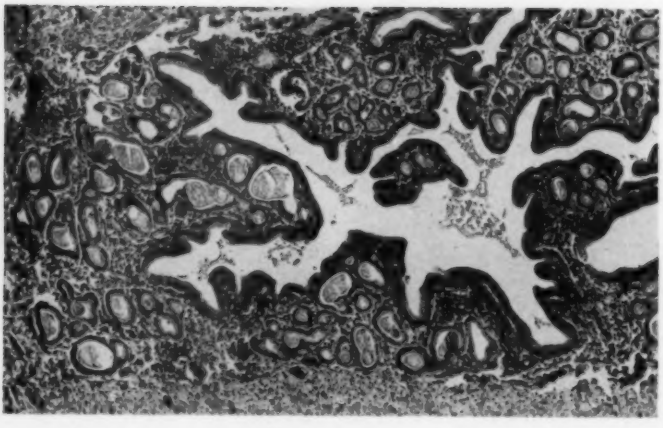


Fig. 11.—Uterus of same tumor-bearing mouse from which Figure 9 was taken. Note extensive proliferation of glands with secretion. Hematoxylin-eosin; reduced to 72% of mag. × 110.

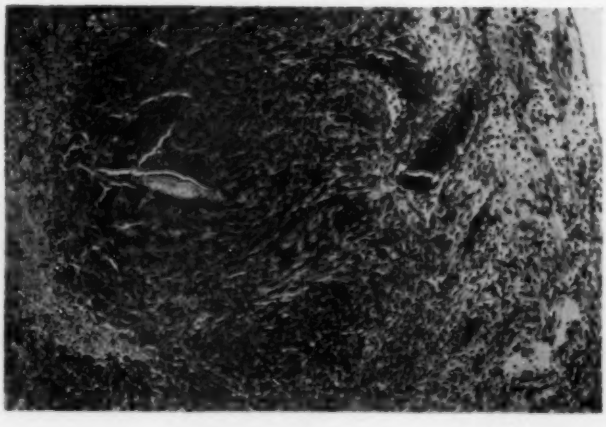


Fig. 12.—Deciduoma in uterine mucosa of a 6-month-old mouse bearing a large Leydig-cell tumor (Strain Rf). Note inflammatory exudate. Hematoxylin-eosin; reduced to 78.5% of mag. × 120.

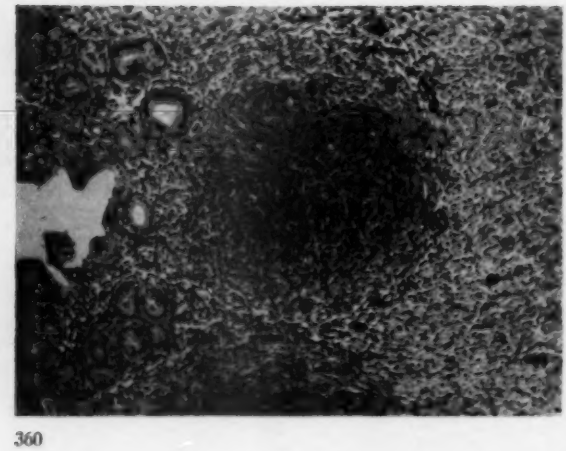


Fig. 13.—Another deciduoma and hypertrophied glands in same mouse as that from which Figure 12 was taken. Hematoxylin-eosin; reduced to 77% of mag. × 120.

Fig. 14. — Cavernous congestion and profound cortical atrophy of the adrenal of a 5-month-old male mouse bearing a large Leydig-cell tumor (Strain Rf). Hematoxylin-eosin; reduced to 80% of mag. × 105.

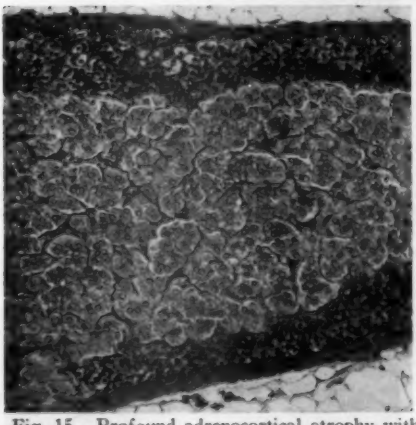


Fig. 15.—Profound adrenocortical atrophy without congestion in a 7-month-old female mouse bearing a large Leydig-cell tumor (Strain Rf). Hematoxylin-eosin; × 150.

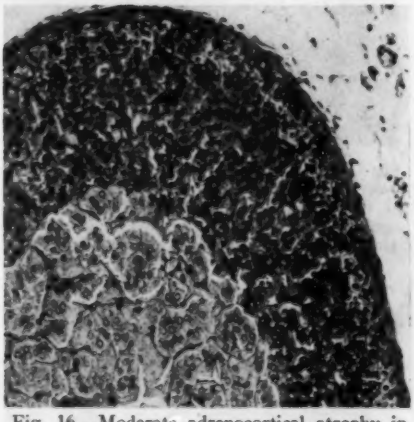


Fig. 16.—Moderate adrenocortical atrophy in a 5-month-old male mouse bearing a Leydig-cell tumor (Strain Rf). Hematoxylin-eosin; × 150.

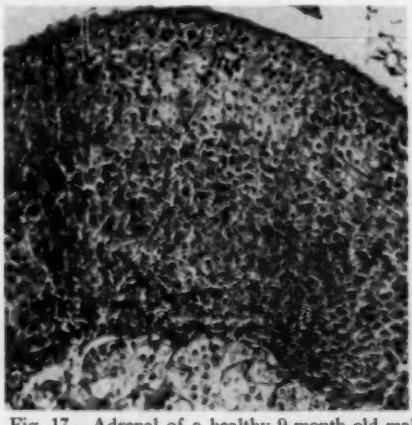


Fig. 17.—Adrenal of a healthy 9-month-old male mouse of the Rf strain. Hematoxylin-eosin; × 150.

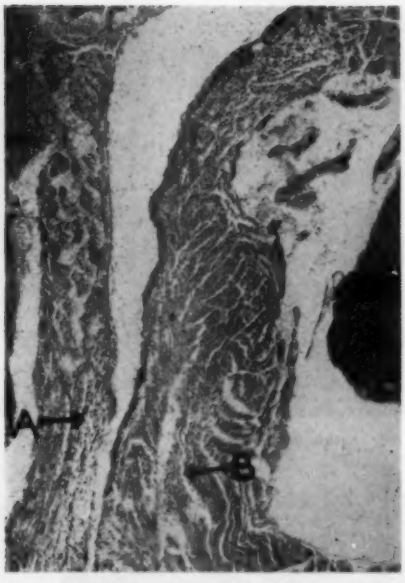


Fig. 18.—Auricular (left) and ventricular myocarditis in a 3-month-old female mouse which died of exsanguinating pleuropericardial hemorrhage. The animal bore a large Leydig-cell tumor (Strain Rf). Hematoxylin-eosin; × 30.

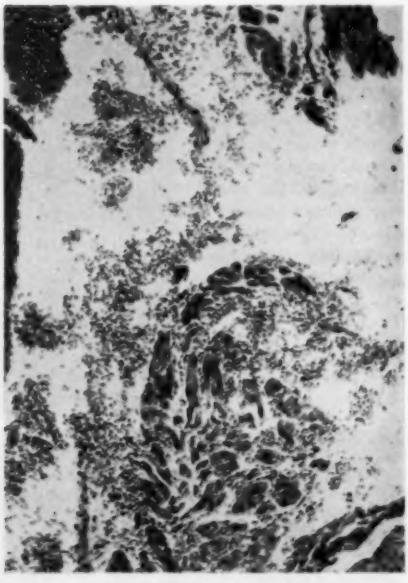


Fig. 19.—Hemorrhage with probable site of rupture of the auricular wall in same mouse from which Figure 18 was taken. Hematoxylin-eosin; × 150.

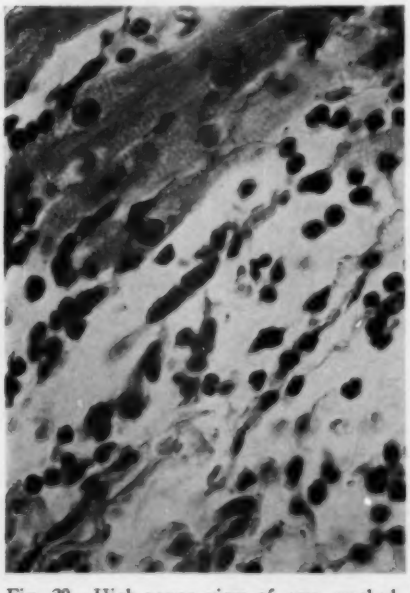


Fig. 20.—High-power view of area marked *A* in Figure 18, showing inflammatory cells, mainly lymphocytes and polymorphonuclear leucocytes. Hematoxylin-eosin; × 600.

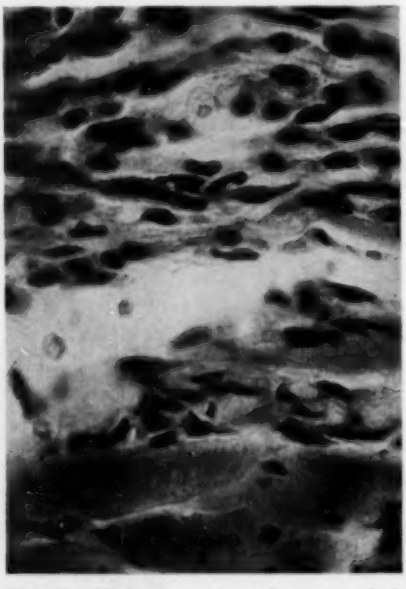
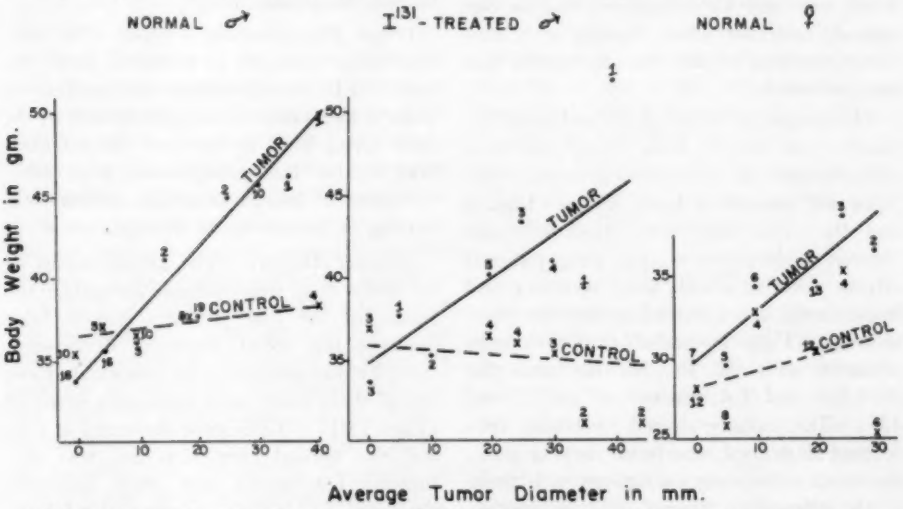


Fig. 21.—High-power view of area marked *B* in Figure 18, showing proliferation of fibroblast-like cells and degeneration of muscle fibers. Hematoxylin-eosin; × 600.

Effect of Tumor on Body Weight.—The Chart illustrates the characteristic increase in body weight with Rf-tumor growth. This

Gonads and Accessory Sex Organs.—The testes of Rf-tumor hosts were usually smaller than normal. Spermatogenesis was



Effect of growth of Leydig-cell tumor (Strain Rf) on body weights of hosts. Dots and crosses represent mean body weights of tumor-bearing and control animals, respectively. Figures indicate number of animals at each point.

was due in part to gain in muscle mass and the weight of the heart, spleen, kidneys, liver, and accessory sex glands (Table 2). Enlargement of the spleens was due to extensive myelopoiesis with predominance of erythropoiesis.

unimpaired, but Leydig cells were very few or absent (Figs. 6 and 7). The seminal vesicles and prostates of Rf- and BALB/c-tumor hosts were usually enlarged and hyperplastic and distended with secretion. Testis size was not markedly affected by

TABLE 2.—*Effect of Leydig-Cell Tumors (Strain Rf) on Body Weights and the Size of Various Organs**

Animals	Males		Females	
	Normal Controls	Tumor-Bearing	Normal Controls	Tumor-Bearing
No. in group	2	2	3†	2†
Body weight, gm.‡	39.2 (38.2-40.2)	45.1 (39.2-51.0)	26.9 (23.6-29.0)	29.7 (28.6-30.8)
Tumor weight, gm.	--	10.2 (10.0-10.4)	--	6.2 (4.4-8.0)
Liver weight, gm.	3.1 (2.4-3.8)	3.5 (3.5-3.6)	1.4 (1.1-1.5)	2.3 (2.1-2.5)
Right kidney weight, gm.	0.34 (0.29-0.40)	0.58 (0.52-0.65)	0.19 (0.18-0.21)	0.36 (0.34-0.38)
Heart weight, gm.	0.26 (0.23-0.29)	0.33 (0.31-0.35)	0.14 (0.14-0.14)	0.36
Right adrenal weight, mg.	3.3 (2.8-3.8)	1.6 (1.4-1.8)	4.0 (3.7-4.4)	1.1
Weight of testes or ovaries, gm.	0.09 (0.09-0.09)	0.07 (0.06-0.08)	0.020 (0.016-0.025)	0.014
Seminal vesicle or uterus weight, gm.	0.21 (0.13-0.30)	0.25 (0.21-0.30)	0.09 (0.08-0.11)	0.42
Ventral prostate weight, gm.	0.045 (0.036-0.054)	0.079 (0.066-0.092)	--	--

* These weights were taken to express quantitatively major weight changes that were obvious in the course of routine autopsies.
† Values from three control and two tumor-bearing animals are included in body, tumor, liver, and kidney weights. The other values are from two controls and one tumor-bearing mouse.
‡ Tumor weight has been subtracted from gross body weight.

the LA tumor, and Leydig cells occurred in normal, or slightly less than normal, numbers. The seminal vesicles of LA-tumor hosts were not hypertrophied, and in one animal, castrated when bearing a 5 mm. tumor, atrophy of the seminal vesicles was not prevented.

The ovaries of hosts of Rf and BALB/c tumors were smaller than normal and were characterized by persistent granulosa follicles and absence of lutein bodies (Figs. 8 and 9). The uteri were thickened and showed moderate to marked progestational effects. Uterine glands were secretory and hyperplastic, often extending into the myometrium (Figs. 10 and 11). Deciduomas occurred with the Rf, but not with the BALB/c and LA, tumors (Figs. 12 and 13). The spindle-shaped decidual cells formed ill-defined whorls of varying sizes, the outer cells being contiguous with those of the submucosal stroma. The deciduomas were usually surrounded by dilated sinuses. Cytoplasm of the decidual cells was scanty and faintly acidophilic. The nuclei were oval and usually contained two nucleoli. Frequently, polymorphonuclear leucocytes were scattered among the decidual cells, and leucocytes often filled the uterine lumina. This unexplained inflammation may have played a role in the induction of deciduomas. The vaginal epithelium was usually multilayered, with mucus-secreting covering cells.

The LA tumor grew in only three females. In these animals, the ovaries were smaller than normal, and the uteri were slightly thickened.

Other Masculinizing Effects.—The kidneys were enlarged in hosts of both Rf- and BALB/c-tumor strains, and sections of kidneys and submaxillary glands were of the male type,‡ even in females. Masculinization became less conspicuous, with other evidences of loss of hormonal secretion occurring in the course of transplantation.

Adrenal Atrophy.—The adrenal cortex of Rf-tumor hosts was markedly atrophic in the course of the first passages, but in later passages this effect gradually diminished. Atrophy was greatest in the fasciculate zone, but all three zones were commonly involved (Figs. 14-17). Cells were decreased in size, and the normal cord structure was disrupted. The cortex was often markedly congested (Fig. 14). Adrenocortical atrophy of less pronounced degree and congestion were also characteristically found in BALB/c-tumor hosts. Adrenals of LA-tumor hosts were less markedly affected. In about half the animals they were partially atrophic, whereas in the rest they were approximately normal.

Exsanguinating Hemorrhage.—Pleuropericardial and, occasionally, periaortic and perirenal "dissecting" hemorrhage was a common cause of death during the first three

‡ References 18, 19.

TABLE 3.—*Exsanguinating Hemorrhage in Mice Bearing Leydig-Cell Tumors (Strain Rf)**

Passage Generation	Males			Females		
	No. Exsang. Hemorrh./ No. Deaths	Days After Graft, Avg.	Tumor Avg. Diam., Mm.	No. Exsang. Hemorrh./ No. Deaths	Days After Graft, Avg.	Tumor Avg. Diam., Mm.
Original, I	8/11 (73%)	160	17	--	--	--
II, III	8/34 (24%)†	166	28	6/42 (13%)†	114	22
IV, V	2/11 (18%)‡	171	18	0/21 (0%)	--	--
VI-X	0/13 (0%)	--	--	0/10 (0%)	--	--

* In this Table, only animals which died 80 days or more after tumor grafting are included, since the earliest death with exsanguinating hemorrhage occurred 82 days after grafting.
† Hemorrhage around kidney in one animal, around tumor in one animal.
‡ Hemorrhage around kidney in one animal.

transplant generations in animals with large Rf tumors (Table 3).

On microscopic examination, the change underlying the exsanguinating hemorrhage appeared to be similar to that described earlier.[20] Sections of the hearts (Figs. 18-21) showed acute or subacute inflammation. In some places, edema occurred with polymorphonuclear leucocytes, and in others monocytes, fibroblasts, and lymphocytes predominated. Myocardial fibers were often widely separated by inflammatory exudate and/or hemorrhage, and some fibers were necrotic or had undergone hyalin degeneration. The inflammation was severest near the auriculoventricular junction and about the origin of the great vessels. It extended into the myocardium, the epicardium, and the walls of great vessels. Three hearts were sectioned serially, and probable points of rupture were located in the walls of auricles and great veins, as indicated by passage of erythrocytes through the walls into the surrounding epicardium (Fig. 19). It is not possible to state with certainty whether the primary change is degenerative or inflammatory. Recent thrombi were noted in the auricles. Inclusion bodies, micro-organisms, and parasites were searched for, but none were identified.

Urine Steroid Analyses.—Two steroid zones were detected on chromatograms of the urine from Rf-tumor hosts. Color tests indicated both of these to be 17-ketosteroids without α,β-unsaturation, and with no carbonyl group attached to ring A of the nucleus. The R_T values (flow rate of individual compounds in heptane-propylene glycol relative to androsterone=1.00 cm/hr.) of zones 1 and 2 were 1.45 and 0.98, respectively. Chromatographic behavior of both zones, and comparisons with chromatograms of mouse and human urine extracts § strongly indicate that zone 1 is identical with an unidentified 17-ketosteroid possessing one additional oxygen-containing group ($C_{19}O_2$ steroid), and zone 2 with androsterone.

§ References 11 and 21. Bloch, E., and Gadsden, E. L.: Unpublished data.

The excretion values per day per mouse were 1.1 mg. of creatinine, 6.5γ of total 17-ketosteroids, 1.6γ of "zone 1, $C_{19}O_2$" steroid, and 1.7γ of androsterone. The results indicate the presence of 40%-50% Zimmerman's chromogenic, nonsteroidal material in the "total 17-ketosteroid" extract. In contrast, normal male LAF_1 mice excrete about 0.03γ of "zone 1, $C_{19}O_2$ steroid," 0.06γ of androsterone, and 0.07γ of etiocholanolone per day, as well as 11-oxygenated-17-ketosteroids.‖

Comment

Comparison of Leydig-Cell Tumors with Luteomas and Tumors of the Adrenal Cortex.—Comparisons of the present studies with those on luteomas and tumors of the adrenal cortex serve to emphasize the physiologic and morphologic similarities of Leydig cells, lutein cells, and cells of the adrenal cortex. Atrophy of Leydig cells of the testes, hypertrophy of the male accessory organs, and masculinization of the kidneys and submaxillary glands are characteristic effects of excessive androgens,[22] and persistence of follicles and absence of corpora lutea are characteristic of ovarian grafts in intact males.¶ Androgens suppress the secretion of gonadotropins,[25] particularly, luteinizing hormone.[26] Thus, the changes in the gonads of animals of both sexes bearing these secretory Leydig-cell tumors could best be explained by excessive androgen levels.

The development of deciduomas in mice bearing tumors is a novel finding. Deciduomas were first induced by Loeb during pregnancy.[27] Deciduomas can be induced only when progesterone levels are high, i. e., during pregnancy, pseudopregnancy, or lactation.[28] Progesterone has been postulated to be formed by testicular, as well as ovarian and adrenocortical, tissues,[29] and has androgenic and mineralocorticoid-like action.# Conversely, androgens will induce

‖ Bloch, E., and Gadsden, E. L.: Unpublished data.

¶ References 23 and 24.

References 30 and 31.

progesterone-like changes in the rabbit uterus.[32] These changes can be brought about either by structural similarities of different compounds or by their interconversion in vivo. Although the uterine gland development in mice with Leydig-cell tumors was similar to that found in mice with transplanted luteomas, deciduomas did not occur with the latter.[16] This may be due either to a qualitative or quantitative difference in the secretions of the two tumor types or to lack of an inciting factor.

Erythropoiesis.—Stimulation of hemopoiesis characteristically follows androgen administration.[33] It occurs constantly in luteoma-bearing mice,[34] which are actually polycythemic. Hepatomegaly also results from androgen administration but may occur to a moderate extent with nonsecretory tumors.[35]

Adrenal Atrophy.—Advanced adrenocortical atrophy occurs with both luteomas and Leydig-cell tumors, and the thymus undergoes atrophy even when Leydig-cell tumors are small and the animals gain weight. However, the chemical data do not show the presence of even small amounts of 11-oxygenated-17-ketosteroids in urines of mice with Leydig-cell tumors, which would be expected if hydrocortisone-like compounds were secreted in quantity. Metabolites of corticosterone would not have been detected by the present methods. Some decrease in the thickness of the adrenal cortex has been induced by administration of androgens[36] and progesterone.[37] These data imply that the release of ACTH is decreased in tumor hosts, but precise knowledge of the steroid which is the immediate cause of this pituitary inhibition is wanting.

Cortisone or corticotropin (ACTH) is stated to cause vacuolation of the cytoplasm, clumping of the granules, and a decrease in the total number of tissue mast cells,[38] while in Leydig-cell tumors there is a characteristic abundance of mast cells. This, too, argues against secretion of glucocorticoids by the Leydig tumor cells.

Steroid Metabolites.—The chemical analyses of excreted steroid metabolites indicate secretion of androgens in large quantity by Leydig-cell tumors. Studies of steroid metabolic pathways in nonrodent species[29] have demonstrated that 17-ketosteroids are metabolites of adrenocortical and testicular hormones. Hormones elaborated by both glands contribute to urinary 11-desoxy-17-ketosteroids, whereas 11-oxygenated-17-ketosteroids are derived mainly from adrenocortical hormones. The predominant metabolites in urine from tumor hosts were the "zone 1, $C_{19}O_2$ steroid" (probably an 11-desoxy-17-ketosteroid) and androsterone. Both are found in much lower concentration in normal mouse urine. The 11-oxygenated-17-ketosteroids and etiocholanolone (which were found in measurable quantities in normal male mouse urine) were not detected in urines from tumor hosts. Absence of the former may perhaps be attributed to the adrenocortical atrophy, but no explanation can be presently advanced for the absence of etiocholanolone.

Exsanguinating Hemorrhage.—This puzzling change was common in tumor hosts during the early passages and deserves special comment. Death from hemorrhage was described in 1.2% of the middle-aged males of the Rf strain.[20] It was commonest during the 14th month of age and affected only males. No etiological agent was detected.[20] In the present series, such hemorrhages were not seen in control animals, most of which were less than a year old when killed. Seventy-three per cent of the males and 13% of the females that survived more than 80 days after grafting of first-generation tumors were affected, and death from hemorrhage occurred in these at 6 to 9 months of age. The data indicate some relation of the disease to male sex hormones. Although spontaneous hemorrhage has been noted only in males of the Rf strain, females bearing these masculinizing tumors were affected. This lesion recalls dissecting aneurysms with transverse rupture of the aorta in man in that both diseases occur most commonly in adult males. In the course of successive passages, when other evidence of androgen secretion by the tu-

mors decreased, the incidence of hemorrhage was markedly reduced, only 18% of the males and none of the females bearing fourth- and fifth-generation transplants being affected. No such hemorrhages were seen after the fifth transplant generation.

Leydig-cell tumors are rich in mast cells, and the latter contain heparin-like and vasoconstricting substances. It is conceivable that mast cells in the tumors play a role in facilitating hemorrhage.

Summary

Three Leydig-cell tumor strains were studied. A highly secreting strain (Rf) was studied in the course of four years in numerous transplant generations; a poorly secreting strain (BALB/c) and a very low-secreting strain (LA) were studied less extensively.

The secondary changes in the Rf-tumor hosts include masculinization; progestational effects, with deciduoma formation in females; adrenal atrophy and obesity in mice of both sexes, and death from exsanguinating pleuropericardial hemorrhage, predominant in males.

The urine of Rf-tumor hosts contains a twenty- to fortyfold increase in two 17-ketosteroids (androsterone and an unidentified $C_{19}O_2$ steroid).

Mast cells are a common feature of both the Rf and the BALB/c tumor strains.

This study emphasizes the similarity in morphology and functional capacity of tumors of interstitial cells of the testis, the corpora lutea of the ovary, and some tumors of the adrenal cortex.

Study of the Rf strain was begun at the Biology Division, Oak Ridge National Laboratory. Dr. Alexander Hollaender, Director of the Biology Division, released living and preserved animals and material needed for continuation of this study. Dr. Elizabeth Fekete furnished animals bearing the BALB/c tumor. Miss Evelyn Gadsden and Mrs. Lena Zompetti gave valuable assistance.

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Effects of Sulfated Polysaccharides on Preestablished Atherosclerosis

Action in the Presence of Continued Cholesterol Feeding

P. CONSTANTINIDES, M.D., Ph.D.
P. SAUNDERS, B.A.
and
A. WOOD, Ph.D., Vancouver, B. C., Canada
With the Technical Assistance of B. Cox

Introduction

It has been shown that heparin, a natural sulfated polysaccharide, prevents the development of atherosclerosis in cholesterol-fed rabbits,* presumably by activating the serum "lipemia-clearing factor" (LCF) and thus promoting enzymatic lipolysis in the blood stream.

It was subsequently demonstrated that sulfated alginic acid (SAA), a synthetic heparinoid, has strong antilipemic and atherosclerosis-preventing properties and that it provokes marked lipid phagocytosis in the spleen, in addition to activating LCF.[1]

The purpose of the present investigation was to find out whether heparin or SAA treatment, instituted after the establishment of atherosclerosis, would arrest the further progress of the lesions, or even initiate their regression, despite continued cholesterol feeding.

Materials and Methods

Ninety-six New Zealand White rabbits (in 11 sets of litter mates) were fed identical amounts of a pelleted 1% cholesterol diet for three months. Periodic serum turbidity and serum total cholesterol determinations were made on all animals throughout the duration of the experiment, as previously described.[4]

At the end of the initial three-month period, the 68 rabbits that achieved with comparable speed the same level of lipemia were selected for further experimentation, whereas the remaining 28 animals were discarded.

The selected 68 rabbits were thereupon divided in four groups of 17 animals each, balanced carefully with regard to sex and litter.

Group I were killed immediately ("early controls").

Group II continued the cholesterol feeding for another two months and were then killed ("late controls").

Groups III and IV also continued the cholesterol feeding for another two months before they were killed, but, in addition, they were given daily heparin and SAA injections, respectively.

During the latter two-month period Groups II, III, and IV were match-fed in triplets. Because of the exceedingly rapid rise of the blood lipid level in all animals during the initial three-month period, cholesterol feeding of Groups II, III, and IV was reduced to four days a week (with a normal diet given during the remaining three days) throughout the last two months of the experiment.

Heparin sodium (Abbott) was given twice daily, subcutaneously, in an amount of 5 mg/kg/injection during the first treatment week, 15 mg/kg/injection during the second and third weeks, and 7.5 mg/kg/injection during the remaining five weeks.

SAA, prepared as previously described,[5] was given once daily, subcutaneously, at a dosage of 5 mg/kg/injection during the first three treatment weeks and 3.5 mg/kg/injection thereafter. Once daily heparin sodium (at 5 mg/kg.) was combined with SAA in Group IV during the first two weeks, but it was discontinued during the remaining six weeks of the injection period.

The following studies were made, in addition to detailed autopsy records:

1. As in previous work,[4] determination of the total aortic atheroma area and the total cholesterol

Submitted for publication May 31, 1956.
From the Anatomy and Animal Nutrition Departments, University of British Columbia.
The results of this study were presented and abstracted in the proceedings of the Banff meeting of the Western Section, National Research Council of Canada (Jan. 25, 1956), and the Milwaukee meeting of the American Association of Anatomists (April 6, 1956).

* References 1, 2, and 3.

content of the aorta (from the valves to the iliac bifurcation)

2. Histological studies on hematoxylin-Sudan red and von Kossa silver nitrate stains of frozen sections of a minute sample from a representative plaque in every aorta of Groups II, III and IV

3. Histological studies on hematoxylin-eosin-stained paraffin sections and hematoxylin-Sudan red-stained frozen sections from standard samples of heart, lung, liver, spleen, kidney, and mesenteric lymph nodes of all groups except Group I, in which the lung, the kidney, and the lymph nodes were not sampled.

The standard sections of the heart were cut through the base of the whole organ, at a level 5 mm. caudal to the free margin of the aortic valves. In assessing coronary atherosclerosis, all arterioles with more than one layer of muscle cells were included in the counts and the percentage of partly and completely occluded coronary vessels noted.

The degrees of renal scarring, visceral xanthomatosis, and splenic atherosclerosis were assessed on an arbitrary scale.

Means and standard errors of the mean (ε) were computed only for values representing objective measurements.

TABLE 1.—*Serum Turbidity, in Klett Units* (Means $\pm\varepsilon$)

Group	Day* 0	Day 80	Day 83	Day 93	Day 108	Day 130
I (early controls)	5.7±0.9	52.1±6.3	Killed			
II (late controls)	5.7±0.9	50.5±7.4		40.4±4.3	37.1±1.3	32.7±4.5
III (heparin)	5.7±0.9	48.5±8.0	Injections started	26.5±3.2	20.7±2.8	16.7±1.5
IV (SAA)	5.7±0.9	49.1±7.9	Injections started	7.6±1.0	8.7±1.4	7.5±0.9

* The days are numbered with reference to the commencement of cholesterol feeding.

TABLE 2.—*Serum Cholesterol (Total)**

Group	Day† 0	Day 80	Day 83	Day 108	Day 130
I (early controls)	34.1±3.9	1446±104	Killed		
II (late controls)	34.1±3.9	1437±126		944±66	873±80
III (heparin)	34.1±3.9	1425±50	Injections started	762±99	648±58
IV (SAA)	34.1±3.9	1412±36	Injections started	449±57	561±42

* Expressed in milligrams per 100 ml. of serum (means± ε)
† The days are numbered with reference to the commencement of cholesterol feeding.

TABLE 3.—*Aorta*

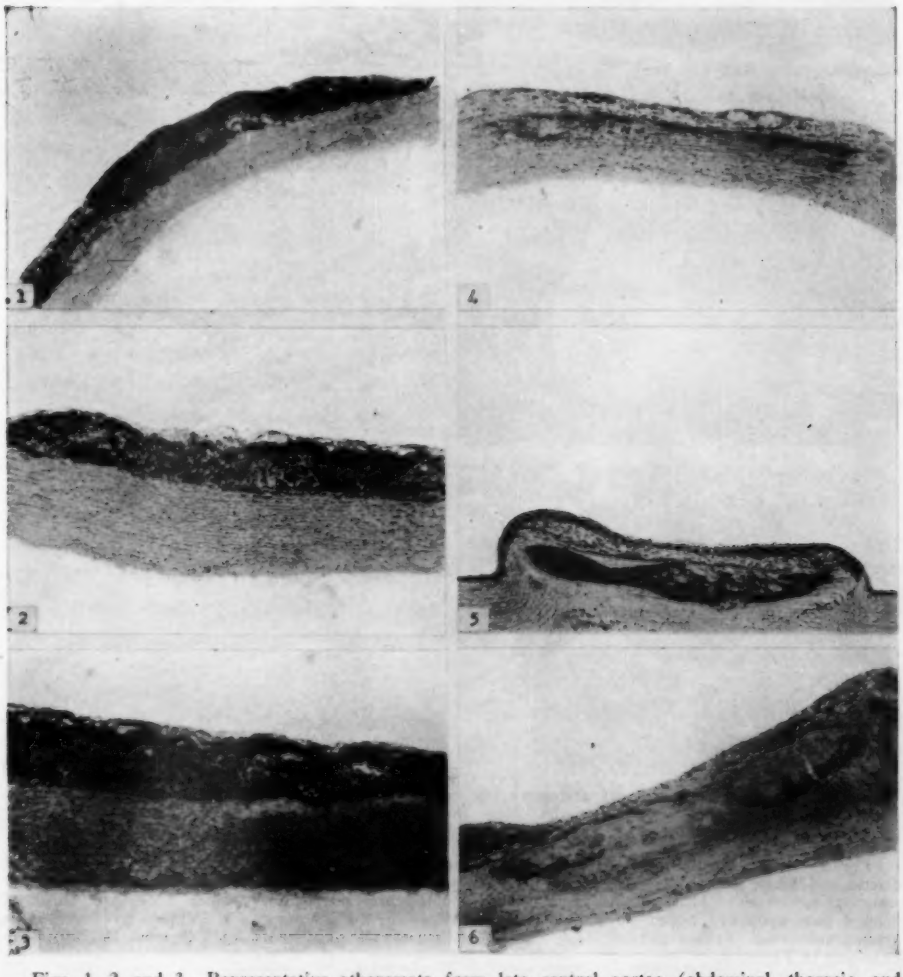
Group	Mean % Aortic Area Occupied by Atheromatous Plaques ± ε	Mean Aortic Cholesterol Content ± ε Mg. Cholest. per Aorta	Mean Aortic Wet Weight, Mg. ± ε	% Animals with Umbilicated Plaques	% Animals with Calcification in Media Under Plaques
I (early controls)	22.3±4.5	7.0±1.3	901±58	0	0
II (late controls)	40.0±5.7	19.1±2.9	1267±82	6	6
III (heparin)	25.6±5.2	13.4±3.6	1133±102	60	47
IV (SAA)	13.7±4.2	7.3±1.8	970±76	53	40

Results

Blood

1. *Serum Turbidity* (Table 1).—A moderate decline of serum turbidity levels occurred in the late controls during the last two months of the experiment, reflecting the reduction of the cholesterol intake in Groups II, III, and IV during that period.

Heparin depressed the serum turbidity to approximately half the late control level,



Figs. 1, 2, and 3.—Representative atheromata from late control aortae (abdominal, thoracic, and arch, respectively). Note the densely packed, largely extracellular, sudanophilic material in those plaques and the absence of calcification in the media. Frozen sections; 15μ; Sudan red-hematoxylin; reduced to 58% of mag. × 45.

Figs. 4, 5, and 6.—Typical lipid-depleted atheromata in aortae of the two treated groups (abdominal, thoracic, and arch, respectively). Note the scarcity of extracellular fat in the plaques and the calcification of the underlying media. Calcification is slight in Figure 4, marked but disintegrating in Figure 5, and mostly replaced by ground substance in Figure 6. Lipophages are seen migrating from the atheroma to the adventitia through gaps in the medial calcinous sheet in Figure 5. Depression of the central plaque area, resulting in "umbilicated" lesions, is visible in Figures 5 and 6. Some sudanophilic material persists in the left margin of the lesion in Figure 6. Frozen sections; 15μ; Sudan red-hematoxylin; reduced to 58% of mag. × 45; same exposure as Figures 1-3.

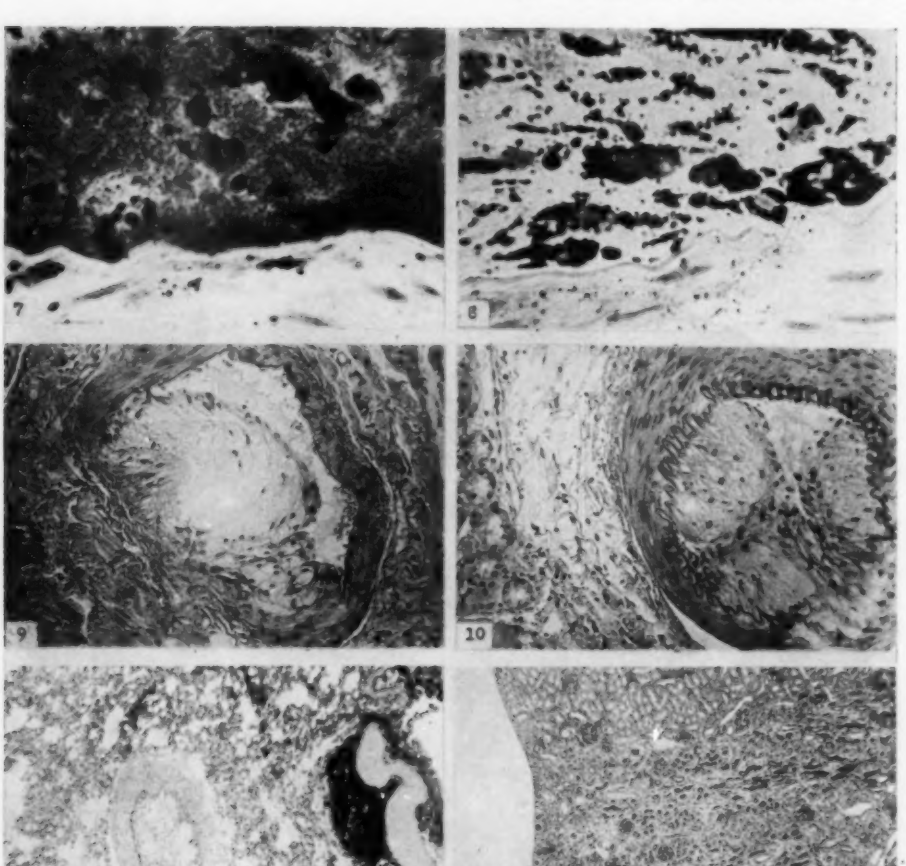


Fig. 7.—Basal portion of a representative late control atheroma. The internal elastic membrane demarcates the atheroma from the media. Note the marked density of the mostly extracellular sudanophilic material. Frozen section; 15μ; Sudan red-hematoxylin; reduced to 58% of mag. × 1250.

Fig. 8.—Basal portion of a typical lipid-depleted atheroma from a treated group. The internal elastic membrane is visible. Note the "dilute" appearance of this lesion, which is due to a very low sudanophilic content. Most of the sudanophilic globules are intracellular, since they are clustered around pale lipophage nuclei. The lipid-free spaces within the atheroma are occupied by a very pale ground substance. Frozen section; 15μ; Sudan red-hematoxylin; reduced to 58% of mag. × 1250; same exposure as Figure 7.

Fig. 9.—Atheromatous coronary artery, early control. Paraffin section; hematoxylin-eosin; reduced to 58% of mag. × 375.

Fig. 10.—Atheromatous renal artery, late control. Paraffin section; hematoxylin-eosin; reduced to 58% of mag. × 375.

Fig. 11.—Atheromatous pulmonary artery, late control. Paraffin section; hematoxylin-eosin; reduced to 58% of mag. × 35.

Fig. 12.—Large wedge-shaped scar in the kidney of a late control. Note the extreme atrophy of the renal tubules, the connective tissue proliferation, and the contraction of the cortical surface. Normal renal parenchyma can be seen near the upper margin of the field. Paraffin section; hematoxylin-eosin; reduced to 58% of mag. × 45.

whereas SAA abolished it altogether. SAA treatment cleared the hyperlipemic sera soon after its commencement and maintained this effect as long as it was given.

2. *Serum Cholesterol* (Table 2).—As in the case of the serum turbidity (and for the same reason), a moderate decline of the serum cholesterol level occurred in the late controls after Day 83.

Heparin had no significant effect on the serum cholesterol level, but SAA depressed it significantly.

Aorta

1. *Total Aorta Atheroma Area* (Table 3).—Three months' cholesterol feeding produced plaques occupying averagely 22% of the aorta surface (early controls). Two months' additional cholesterol feeding expanded the total plaque area to 40% of the aorta surface. Both heparin and SAA arrested this expansion of the total atheroma area.

2. *Aortic Cholesterol Content and Aortic Wet Weight* (Table 3).—Three months' cholesterol feeding caused an average deposition of 7 mg. of cholesterol in each aorta. Two months' additional cholesterol feeding increased this to 19 mg. per aorta. Heparin had no significant effect on the increment of the cholesterol deposition, but SAA prevented it completely.

Furthermore, a comparison of the early and late controls shows that two months' additional cholesterol feeding caused a moderate elevation of the aortic wet weight. This elevation was completely suppressed by SAA and only partly inhibited by heparin.

3. *Gross Anatomic Features of the Aortic Plaques* (Table 3).— Grossly, the plaques of the majority of the two treated groups were flat, and they frequently presented a "moth-eaten" appearance, with marked central shrinking and loss of yellow lipid. In extreme cases, such plaques were concave, resembling saucers or shallow pits. The media under most of these umbilicated lesions contained white, calcinous deposits.

The plaques of the majority of the two control groups were convex and elevated. None of the early control and only one of the late control animals exhibited umbilicated lesions with calcinous medial deposits.

4. *Microscopic Features of the Aortic Plaques* (Figs. 1-8).—Histologically, the umbilicated plaques that were seen in many heparin- and SAA-treated animals proved extremely poor in sudanophilic lipids, the tiny droplets of fat in them being entirely intracellular, i. e., engulfed by numerous scavenger cells. In most, though not all, instances the media directly underneath such umbilicated plaques showed calcification, as evidenced by the von Kossa silver nitrate stain.

Various stages of disintegration and resorption of these calcium deposits, occasionally involving multinucleated giant cells, could be observed.

In striking contrast, the lesions of almost all control animals were tightly packed with mostly extracellular sudanophilic fat, scav-

TABLE 4.—*Heart and Lung*

Group	Mean Total Number of Coronary Vessels Encountered per Section ± e	Mean % Atheromatous Coronary Vessels ± e	Mean % Completely Occluded Coronary Vessels ± e	Mean % Atheromatous Pulmonary Vessels ± e	Mean Degree of Pulmonary Xanthomatosis
I (early controls)	26.6±1.9	23.2±4.3	8.4±0.1	----	----
II (late controls)	22.8±2.9	42.3±4.2	30.1±1.1	24.2±4.0	0.8
III (heparin)	26.5±1.9	31.8±4.0	16.3±3.3	4.6±2.2	0.4
IV (SAA)	27.4±2.1	19.3±3.4	10.4±2.2	2.2±0.9	1.3

TABLE 5.—*Kidney*

Group	% Animals with Scars	Mean Degree of Scarring (Arbitrary Scale)	% Animals with Fresh Necrosis	% Animals with Interstitial Xanthomatosis	% Animals with Glomerular Foam Cells
II (late controls)	71	1.7	53	53	6
III (heparin)	33	0.7	27	7	0
IV (SAA)	27	0.3	0	13	47

enger cells being very scarce and frequently in the process of cytolysis.

Other Organs

1. *Heart* (Table 4, Fig. 9).—SAA arrested the expansion of the atheromatous process in the coronary bed, as judged by both the number of atheroma-bearing and the percentage of completely occluded coronary arteries encountered in the standard sections through the base of the heart.

Heparin simulated SAA in arresting the increment of coronary occlusion, but it had only a borderline effect on the increment of the number of atheromatous vessels.

Despite the complete occlusion of numerous coronary arteries of all calibers, no massive myocardial infarctions were seen. This was probably due to the gradual character of the occlusive process and to the speedy development of collateral circulation. Multiple minute foci of myocardial necrosis, accompanied by mononuclear infiltration, were, however, regularly observed in both control groups. These lesions were almost absent from the hearts of the two treated groups.

Moderate interstitial xanthomatosis appeared in the myocardium of three SAA-treated animals.

2. *Lung* (Table 4; Fig. 11).—Both heparin and SAA markedly inhibited the progress of pulmonary atherosclerosis.

Mild xanthomatosis appeared in the lungs of the late controls, particularly in the pleura and the subpleural parenchyma. Heparin decreased, whereas SAA increased, this reaction.

Rarely, large foam cells were observed floating free within the lumen of pulmonary vessels of the late controls and the two treated groups, without any apparent group predilection.

3. *Spleen* (Table 6).—Heparin had no effect on the weight or the gross appearance of this organ.

The spleens of the SAA-treated animals, however, increased to twice the size of the late control organs, and their external and cut surfaces frequently presented a yellow peppered appearance.

Microscopic examination revealed a mild degree of xanthomatosis in the red pulp of

TABLE 6.—*Spleen, Mesenteric Lymph Nodes, Adrenals*

Group	Mean Degree of Splenic Atherosclerosis (Arbitrary Scale)	Mean Degree of Splenic Xanthomatosis (Arbitrary Scale)	Mean Splenic Wet Weight ± σ (Mg./Kg. Terminal Body Wt.)	Mean Degree of Lymph Node Xanthomatosis (Arbitrary Scale)	Mean Left Adrenal Wet Weight ± σ, Mg./Kg. Terminal Body Wt.
I (early controls)	1.8	0.8	----	----	----
II (late controls)	2.4	2.1	620±240	0.6	139±13.4
III (heparin)	1.6	0.3	570±210	0.6	132± 9.0
IV (SAA)	1.7	2.7	1190±180	1.3	116± 6.1

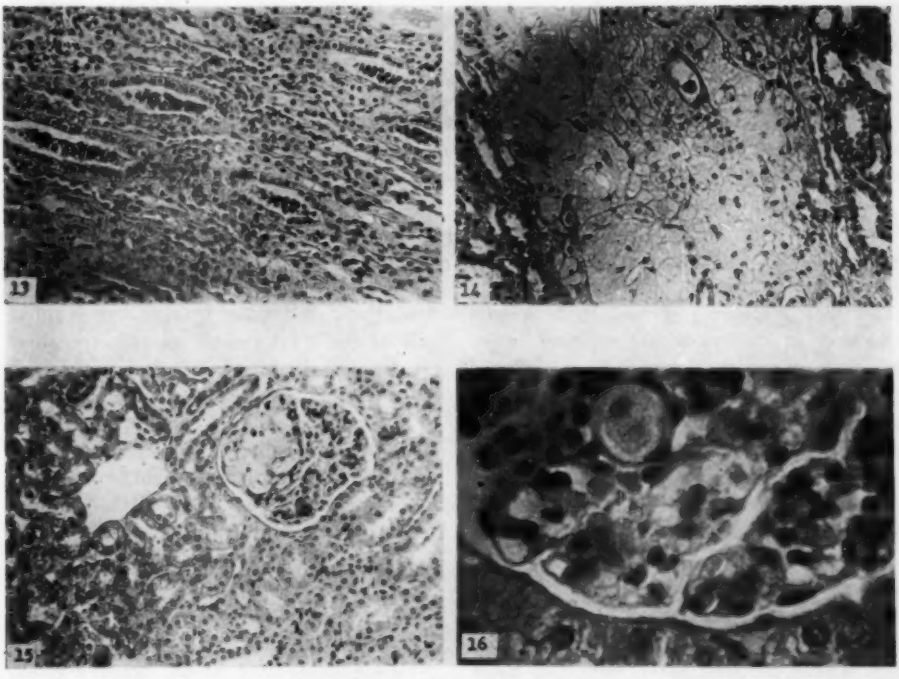


Fig. 13.—Epithelial casts in the collecting tubules of a late control kidney. Paraffin section; hematoxylin-eosin; reduced to 58% of mag. × 375.

Fig. 14.—Xanthomatous stroma in the kidney of a late control. Paraffin section; hematoxylin-eosin; reduced to 58% of mag. × 375.

Fig. 15.—A cluster of foam cells occupying a glomerular lobe in the kidney of an SAA-treated rabbit. Note the normal parenchyma surrounding the glomerulus. Paraffin section; hematoxylin-eosin; reduced to 58% of mag. × 375.

Fig. 16.—A large solitary foam cell lodged in a glomerular capillary of an SAA-treated animal. The parietal layer of Bowman's capsule is visible near the lower margin of the field. Paraffin section; hematoxylin-eosin; reduced to 58% of mag. × 1250.

the early controls, increasing to severe in the late control and the SAA-treated group. Foam-cell formation was distinctly suppressed by heparin in this organ.

Both heparin and SAA inhibited the moderate progress of splenic atherosclerosis.

4. *Mesenteric Lymph Nodes* (Table 6).—Slight xanthomatosis occurred in the medullary trabeculae of the late control lymph nodes. Foam cells were encountered both fixed in the medullary cell cords and free in the penetrating sinuses. Heparin did not influence this reaction, but SAA increased it.

5. *Kidney* (Table 5; Figs. 10 and 12-16).—Severe occlusive atherosclerosis of major renal artery branches in the late controls caused massive destruction of the renal parenchyma, leading to a typical "scarred contracted kidney" in this group. Grossly visible, large wedge-shaped scars dominated the picture and accounted for the multiple pitting of the kidney surface. Histological study showed extensive atrophy and disappearance of glomeruli and tubules, along with marked fibrous replacement.

Desquamation of tubular epithelium and tubular dilatation were seen at the borders of the fibrotic areas, whereas patches of necrosis and dense mononuclear infiltration (presumably corresponding to fresh infarcts) were scattered between them. Finally, xanthomatous streaks occurred regularly in the interstitial stroma of the corticomedullary zone. In several instances,

the tips of the wedge-shaped scars were continuous with these xanthomatous streaks.

SAA, and to a slightly less pronounced extent heparin, protected the renal parenchyma from destruction secondary to atherosclerosis and from the interstitial xanthomatosis.

SAA also caused the appearance of lipid-laden foam cells in the renal glomeruli of approximately half the treated animals. These foam cells might be of embolic origin, since they sometimes seemed to be lodged singly or in clusters within capillary loops. In most cases the glomeruli so afflicted (which could be seen grossly as yellow points) were hypertrophic and contained several patent capillary loops—a fact that probably accounted for the remarkable normalcy of the surrounding tubules. Less than 30% of all glomeruli showed marked foam-cell involvement in the majority of the animals that showed this reaction. Marked foam-cell involvement of all glomeruli was, however, evident in the two animals that died within the first three weeks of SAA treatment.

None of the heparin-treated and only one of the late control animals presented glomerular foam cells.

6. *Liver.*—There was the usual heavy fatty infiltration of this organ in all four groups. Almost all of the fat was contained in the liver cord cells (which frequently showed double nuclei), very little of it appearing in the Kupffer cells. Patches of cytolysis were seen more frequently in the late control and the heparin-treated group than in the early control or the SAA-treated animals. Beginning centrilobular cirrhosis was evident in two late controls and in one heparin-treated rabbit, but it was absent from the early controls and the SAA-treated animals.

There was no significant variation in the degree of fat deposition in the liver cord cells of the four groups. Fat droplets were encountered less frequently in the Kupffer cells of the early controls than in those of the late controls or the two treated groups. Among the last three groups, the Kupffer cells of the SAA-treated animals contained fat droplets slightly more frequently than those of the late controls or the heparin-treated rabbits.

7. *Adrenal* (Table 6).—There was no significant difference in the weight or the gross appearance and consistency of the adrenals of the three groups sampled.

Mortality, Growth, and Clinical Condition (Table 7)

No deaths occurred in any of the two control groups.

Two animals died in the heparin group and two in the SAA-treated group, during the first three weeks of treatment. Autopsy findings indicated that the probable cause of death was massive internal bleeding in the heparin-treated and glomerular blockage in the SAA-treated rabbits. Reduction of the heparin dosage from twice 15 to twice 7.5 mg/kg. daily and of the SAA dosage from 5 to 3.5 mg/kg. daily was apparently responsible for the absence of further deaths in both experimental groups during the remaining five weeks of treatment.

Thus, the experiment was terminated with

TABLE 7.—*Summary of Body Weights*

	Mean Initial Body Wt. (Kg.) ± σ	Mean Pretreatment Body Wt. (Kg.) ± σ	Mean Terminal Body Wt. (Kg.) ±
I (early controls)	2.66±0.061	2.85±0.05	.....
II (late controls)	2.70±0.063	2.90±0.054	3.20±0.075
III (heparin)	2.72±0.060	2.90±0.045	3.04±0.066
IV (SAA)	2.55±0.065	2.80±0.052	2.90±0.095

17 animals in each of the two control groups and with 15 animals in each of the two injected groups.

All animals surviving at term were in good clinical condition and singularly free from pneumonia, coccidiosis, enteritis, ear mites, and other common infections.

The animals of all four groups gained weight at the same rate before injections were started. Treatment with both heparin and SAA diminished the growth rate by about the same amount, but it did not arrest growth or lead to any catabolism.

Comment

Quantitative evidence was obtained that both heparin and SAA arrested the expansion of the preestablished atheroma area in the aorta and the visceral vascular beds, in the presence of continued high cholesterol intake. Only SAA, however, arrested the further deposition of cholesterol in the aortic lesions. The generally greater atherostatic effect of SAA (as against heparin) appeared to be in line with its superior antilipemic effect. It will be recalled that SAA depressed the hypercholesteremia and completely abolished the serum turbidity, whereas heparin did not affect the former significantly and only partially depressed the latter, under present conditions.

It is of interest that heparin has similarly failed to affect serum turbidity or serum cholesterol when applied after the induction of heavy lipemia and hypercholesteremia by protracted cortisone treatment in rabbits.[6] On the other hand, heparin has been found to prevent the development of hyperlipemia [2] and partially to inhibit the blood cholesterol rise † when combined with cholesterol feeding from the start, in rabbits. It would thus seem that the effects of heparin on rabbit lipemia depend, among other things, on whether this principle was applied before or after the development of a prolonged hyperlipemia. The reasons for such a discrepancy are at present unknown. It may well be that protracted hyperlipemia leads to the gradual exhaustion of tissue or blood co-factors necessary for the production of the "lipemia-clearing factor" (LCF) by heparin, or that the chylomicra and lipoproteins of hyperlipemias of long standing became resistant to the clearing action of heparin.

The appearance of umbilicated, lipid-depleted plaques in the two groups receiving antilipemic treatment suggests that SAA and heparin also initiated the regression of several preestablished atheromata.

If it is assumed that the lipids in the blood stream are in dynamic equilibrium with those in the plaques (as has been postulated for the relationships between plasma and liver cholesterol or plasma and erythrocyte cholesterol [7]), it could be expected that a sustained reduction of the plasma lipid concentration would provoke an egress of lipids from plaques, provided the membrane separating the two is sufficiently thin to permit such a transfer. It is further possible that the heparinoid treatment, apart from any indirect effects through the blood lipids, might attack the lipids within the plaques directly, either through local activation of LCF in the intimal tissue or through facilitation of lipid phagocytosis by the plaques' own macrophages. Finally, though rather remote, the possibility cannot be excluded that some of the umbilicated, lipid-depleted plaques were not modified preestablished lesions but new lesions of a very rudimentary and arrested character.

We have, at the moment, no explanation for the finding of a few umbilicated lesions and medial calcifications in one late control animal. This phenomenon could be related to the moderate decline of lipemia caused by the dietary cholesterol restriction in all groups after Day 83, or else it suggests that sporadic regression may occasionally occur even in the presence of continued hyperlipemia.

It is evident that the final evaluation of these qualitative plaque changes will have to await the results of experiments designed to study the pattern of the spontaneous regression that follows discontinuation of

† References 2 and 3.

cholesterol feeding and the effects of sulfonated polysaccharides on such regression. It may be well to keep in mind that absolute proof of induced regression will ultimately rest on direct in vivo observations of the fate of a given atheroma or of homologous atheromata in the same animal.

The mechanism leading to calcifications of the media under most umbilicated, lipid-depleted lesions is similarly open to speculation. Since these calcifications were found almost exclusively in the two treated groups, it is probable that they represented calcium soaps formed by free fatty acids released from hydrolyzed triglycerides, as they percolated into the media. Free fatty acids could arise from the hydrolysis of neutral fats in the lesions or in the blood stream. Since recent in vitro experiments have shown that the heparin-induced LCF clears hyperlipemic plasma by splitting triglycerides into glycerin [8] and free fatty acids,[9] and since it has been demonstrated that preestablished plaques have an increased permeability,[10] it is likely that plasma lipids constituted a substantial source of the observed calcifications. Nevertheless, alternative explanations will have to be ruled out by further experimentation. The fact that even the animals that died early in the course of polysaccharide injections presented medial calcifications indicates that this phenomenon occurs very rapidly, perhaps within the first few days of treatment.

As far as the reaction of the reticuloendothelial system is concerned, the present data lead to the conclusion that lipid phagocytosis by tissue macrophages occurs physiologically in rabbits subjected to very prolonged hyperlipemia and that SAA increases this response (in the spleen, the mesenteric lymph nodes and the lungs, but not in the renal stroma or the liver), whereas heparin suppresses it in almost all tissues. Thus, the strikingly greater antilipemic activity of SAA, as compared with heparin, might be due to the fact that SAA attacks blood lipids by stimulating both enzymatic lipolysis and cellular lipophagia, whereas heparin acts only through the former mechanism.

The fact that the SAA-induced splenic enlargement was much less prominent in the present experiment than in a previous study [4] (in which much greater amounts of SAA were used) indicates that this reaction is proportional to the SAA dosage level. On the other hand, it is possible that the brief combination of heparin and SAA during the initial phase of the injection period was partly responsible for the diminished splenic response.

The glomerular foam cells seen in the kidneys of many SAA-treated and one late control animal are possibly of embolic origin, although this will have to be proved by the actual demonstration of foam cells in the blood stream of animals showing such lesions. While it is true that free floating foam cells were encountered sporadically within the lumen of pulmonary vessels of a few control and treated animals, they might have been artificially detached from atheromata during the manipulation of lung specimens prior to fixation. Pending future investigation, there are indications that this phenomenon reaches a maximum in the first few days of SAA treatment, when the excess lipids are "swept off" the blood stream.

On the whole, the results of this study are of appreciable theoretical interest inasmuch as they show that the growth of preestablished mammalian atheromata can be completely arrested and regressive changes initiated, despite continued high lipid intake. They thus contribute to the establishment of a rationale for the heparin treatment of those types of human atherosclerosis that are demonstrably associated with sustained hyperlipemia or hypercholesteremia. On the other hand, it is evident that clinical trials with the much more potent synthetic heparinoids, such as SAA, will have to be postponed until the side-effects of these substances are thoroughly understood and brought under control.

It is hoped that further research along those lines will eventually lead to com-

pounds or combinations of compounds exhibiting maximal atherosclerosis-arresting activity with minimal side-effects.

Summary

Heparin and sulfated alginic acid (SAA) treatment was instituted in rabbits some time after the development of cholesterol atherosclerosis and was carried on in the presence of continued high cholesterol intake.

Under the conditions of this experiment, SAA displayed considerably greater antilipemic activity than heparin. SAA abolished the lactescence of the serum and reduced the hypercholesteremia, whereas heparin reduced the lactescence and had no significant effect on the hypercholesteremia.

Both heparin and SAA arrested the expansion of the preestablished atheroma area in the aorta and visceral arteries, but only SAA arrested the further deposition of cholesterol in the aortic lesions.

Qualitative changes suggestive of incipient regression appeared in several aortic plaques of heparin- as well as SAA-treated animals. These changes consisted of central shrinkage, histological lipid depletion, and calcification of the underlying media.

Both heparin and SAA inhibited xanthoma formation in the interstitial stroma of the kidney and protected the parenchyma of this organ from destruction secondary to atherosclerosis.

Heparin suppressed, whereas SAA increased, the appearance of foam cells in the spleen, the mesenteric lymph nodes, and the renal glomeruli.

This investigation was supported principally by a grant from the Life Insurance Medical Research Fund, New York, with some financial assistance from the National Research Council of Canada. Heparin sodium was supplied by Abbott Laboratories, Chicago, and sodium alginate SS/LF (the raw material for the preparation of SAA), by Alginate Industries, Ltd., London, England.

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Morphogenesis of Calcareous Deposition in the Adrenal Glands of the Cat

CAPT. REUBEN EISENSTEIN (MC), U. S. Army

The occurrence of spontaneous disease in laboratory animals is of paramount importance to research in biology, not only because such disease can kill or cause definite clinical signs in experimental animals but also because such disorders can cause alterations of tissues which may erroneously be interpreted as a result of experimental treatment. The object of this study is to describe the morphogenesis of a lesion that was found in many cats necropsied in our laboratories.

Ross and associates [5] (1955) stated that the adrenal glands of many monkeys and cats (also, rarely, of dogs) contained corpora calcificantia, mainly within the cortex or at the corticomedullary junction, as well as finely granular calcareous deposits which were similar in distribution. These investigators were particularly impressed by the morphologic alterations, aside from calcification, which they saw in the adrenal glands of cats, stating that few adrenal glands of cats could be designated as normal; and they believed that the abnormalities might be a sequel of a hitherto unidentified virus infection of cats.

Methods

Forty-five cats, ranging in size from that of the newborn to 3.4 kg. in weight, were examined. They were animals collected by dealers, having no record of any form of vaccination. Neither the clinical nor the dietary history was known, and it was not possible to determine the exact age; they had probably been exposed to a variety of infectious diseases. Animals were anesthetized with intraperitoneal injections of pentobarbital, after which both adrenal glands were removed for immediate fixation in neutral buffered 10% formalin. Paraffin sections were stained with hematoxylin and eosin, toluidine blue, and the periodic acid-Schiff reagent. Frozen sections, cut 5μ to 10μ thick, were stained with Sudan IV, and sections were also prepared with the Schultz method for cholesterol, with the Ashbel-Seligman technique (Ashbel and Seligman,[1] 1949), and with the Feulgen plasmal reaction, and were also examined by polarized light for birefringence. The tests on frozen sections were repeated on other, adjacent sections after they had been immersed in acetone overnight; this resulted in negative reactions in all but the Ashbel-Seligman procedure, in which the intensity of coloration was diminished. Although it was at one time suggested that a battery of procedures similar to the above is capable of localizing intracellular corticosteroids, Feldman [9] (1950) gave convincing evidence that there are no histochemical reactions that are specific for corticosteroids. The Schultz method is evidently specific for cholesterol and its esters (Gomori,[6] 1952). The Sudan dyes stain lipids, and the periodic acid-Schiff reaction (with some reservations) indicates 1-2 glycol linkages. The latter reaction, and the toluidine-blue reaction for metachromatic substances, indicate the presence of one of the acid mucopolysaccharides. The Ashbel-Seligman and Feulgen plasmal reactions probably demonstrate lipid aldehydes, which are formed in the tissues from unsaturated fatty acids during formalin fixation (Gomori,[6] 1952; Karnovsky and Deane,[13] 1955).

Results

Two types of abnormality, which seem to be morphogenetically related, were found, namely, fatty vacuolation of the cells of the adrenal cortex and calcification.

Fatty Vacuolation of the Adrenal Cortex.—The normal mammalian adrenal cortex, particularly the outer part of the zona fasciculata, consists mostly of large, fatty cells called spongiocytes (Bailey,[10] 1948; Bennett,[2] 1940). Judging from observations on what are considered to be normal adrenal

Submitted for publication June 20, 1956.

Pathology Branch, Directorate of Medical Research, Army Chemical Center, Md.

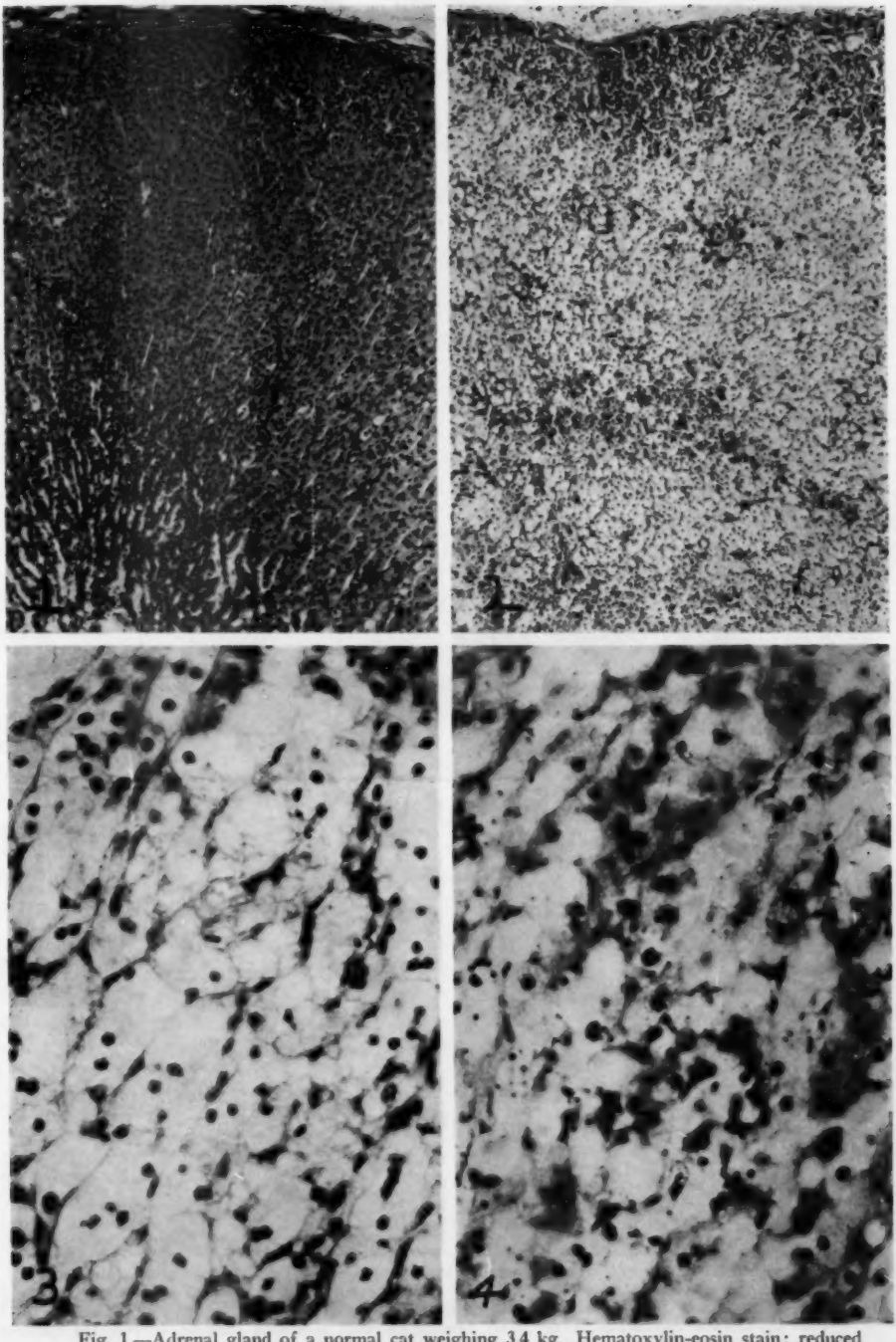

Fig. 1.—Adrenal gland of a normal cat weighing 3.4 kg. Hematoxylin-eosin stain; reduced to 92% of mag. × 92.

Fig. 2.—Adrenal gland, cat, showing marked fatty metamorphosis of the zona fasciculata. Hematoxylin-eosin stain; reduced to 92% of mag. × 92.

Fig. 3.—Higher magnification of adrenal gland shown in Figure 2. Note swollen, vacuolated, multinucleated cells with pyknotic nuclei, and extracellular droplets of fat. Hematoxylin-eosin stain; reduced to 92% of mag. × 425.

Fig. 4.—Deposits of finely granular calcium surrounded by a zone of fatty metamorphosis. The matrix of the calcareous deposits is metachromatic. Toluidine blue; reduced to 92% of mag. × 425.

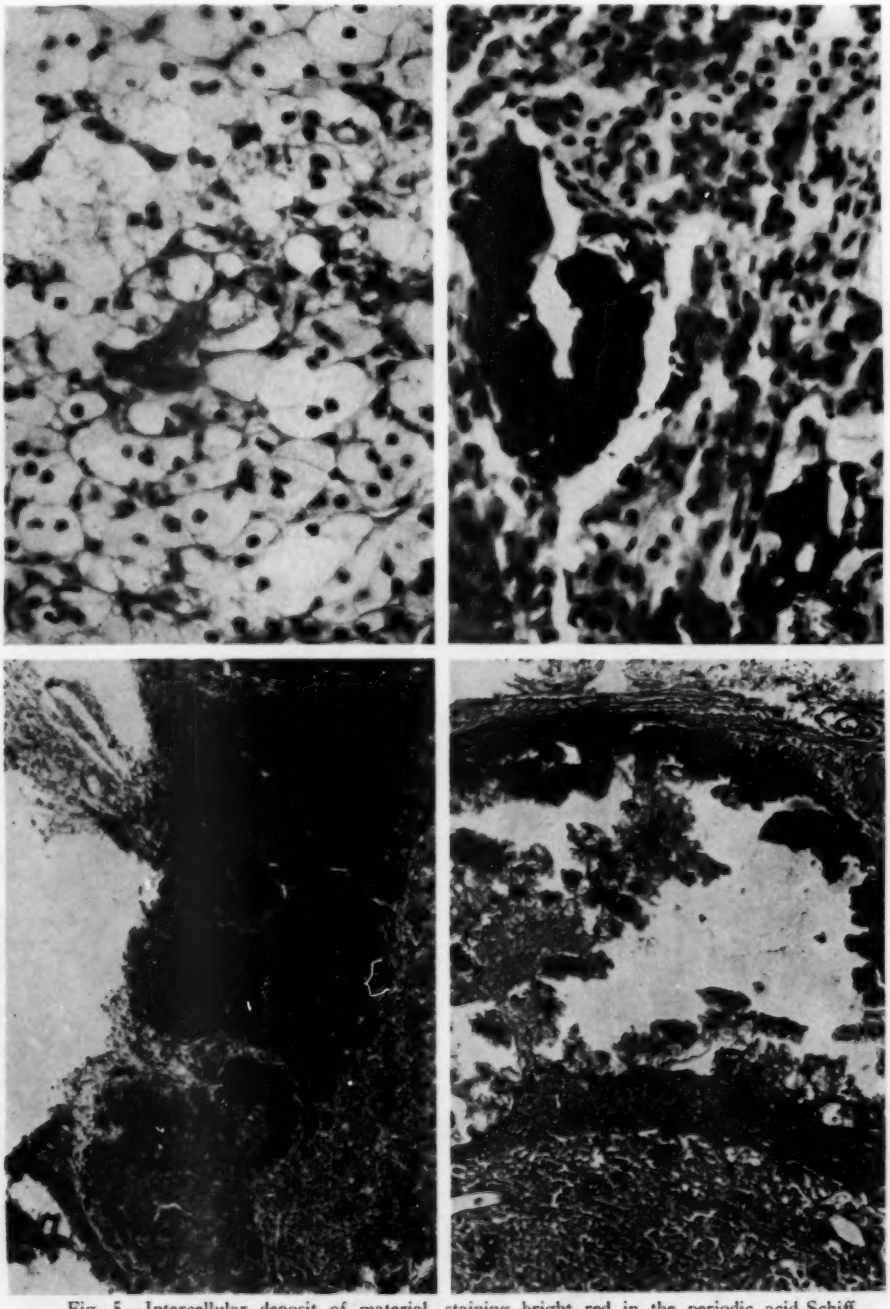

Fig. 5.—Intercellular deposit of material, staining bright red in the periodic acid-Schiff reaction, in the adrenal gland of an adult cat. Note the surrounding zone of fatty metamorphosis. Periodic-acid Schiff reaction; reduced to 92% of mag. × 425.

Fig. 6.—Corpus calcificans in the zona fasciculata with a fibrous capsule, surrounded by normal adrenal cortical cells. Hematoxylin-eosin stain; reduced to 92% of mag. × 425.

Fig. 7.—Adrenal cortex, cat. The large black mass in the upper part of the picture is a group of strongly sudanophilic cells. In the lower part of the picture, the dark mass is a corpus calcificans, colored deep purple by hematoxylin; within its paler center are small sudanophilic droplets, which show as black particles in the photograph. Sudan IV counterstained with hematoxylin; reduced to 92% of mag. × 92.

Fig. 8.—Adrenal cortex, showing the size which the calcareous bodies may attain. The mass involved most of the thickness of the adrenal cortex. Much of the calcium has fallen out during cutting. Hematoxylin-eosin stain; reduced to 92% of mag. × 92.

glands of the cat, and from available illustrations in articles on normal structure (Bennett[2] Nicander[6]), the cytologic appearances described here lie outside the range of normal variation in structure.

Fatty metamorphosis occurred in one or all of the zones of the adrenal cortex. The cells were extremely vacuolated and enlarged, with only fine threads of cytoplasm visible in hematoxylin-eosin preparations. Many of the nuclei were pyknotic. The intracytoplasmic vacuoles were found to be laden with acetone-soluble lipids, including cholesterol, doubly refractile lipids, and substances deeply colored in the Feulgen plasmal and Ashbel-Seligman reactions. Cytoplasmic masses with two, three, or more nuclei were numerous, resembling the lesion of lipodiastema formation in the liver. Some areas of the adrenal cortex contained extracellular droplets of fat.

Calcareous Depositions.—Focalized calcareous bodies (corpora calcificantia of Ross and associates) were found in large masses that sometimes involved the entire thickness of the cortex, and some of the bodies possessed a fibrous capsule. Calcification also appeared as a fine extracellar granular deposition. Ross and associates[8] showed by microincineration that the corpora calcificantia consisted chiefly of the ash residue of calcium and/or magnesium, together with traces of iron. In the present study, granular calcium in the adrenal cortex of cats was always found to be surrounded by a zone of fatty metamorphosis, but the corpora calcificantia with fibrous capsules were usually surrounded by normal cortical cells. Calcium was deposited in a matrix of material that colored bright red in periodic acid-Schiff preparations and was metachromatic. In a few cases calcareous bodies were found to contain neutral sudanophilic fat.

These histologic alterations appeared to be related to the age and weight of the cat, for neither fatty metamorphosis nor calcareous bodies were found in animals that weighed less than 1 kg. Newborn kittens were free of any lesions in the adrenal glands. However, some of the young animals (weighing less than 1 kg.) possessed occasional fatty vacuolated cells in the adrenal cortex. No difference in the incidence of lesions between the sexes could be detected.

Of the 45 cats in this study, 27 weighed over 1000 gm. Of these 27 cats, 12 showed advanced fatty metamorphosis, involving the entire cortical width of the zona glomerulosa, or at least half the thickness of the zona fasiculata. The zona reticularis was only rarely involved with fatty metamorphosis. The glands of 5 of the 27 animals had large islands of such cells, and in 6 there was an occasional cell, leaving 4 of the 27 cats weighing over 1 kg. which had histologically normal glands. Fatty metamorphosis of the zona fasciculata usually involved the outer part of the zone, which normally is richer in lipids than the inner part. The pattern of fatty metamorphosis was always similar in the two adrenal glands of the same cat.

Calcification was found most frequently in the zona fasciculata, but in many cases the other zones were also found to contain calcareous bodies. The adrenal cortices of 10 of 27 cats weighing over 1000 gm. (32%) were found to contain corpora calcificantia or granular calcifications, which were bilateral in 6 of the 10 animals. Serial sections were made in only one case, and calcification was found that had been missed in a single section. Hence, it is possible that the incidence of calcification was higher in our series, and perhaps also in that of Ross and co-workers, and that it may always be bilateral.

Comment

Rubin and Howard[9] presented the idea that calcium in tissue virtually occurs always in a matrix of chondroitin sulfate, which is colored bright red in tissue sections after treatment with periodic acid-Schiff reagent and stains metachromatically with toluidine blue. Such a matrix has been found to be associated with the calcareous deposits in the adrenal glands of cats. Thus, the morphogenesis of pathologic calcification in the adrenal cortex of the cat can be reconstructed

as follows: The cell walls of the swollen, lipid-laden cortical cells become ruptured to form multinucleated masses of protoplasm, or simply to fill up tissue spaces with fat, which eventually may be carried off in the circulatory system. Metachromatic mucopolysaccharides are then laid down, on or with which calcium is deposited in finely granular form. As such a process continues, the concretions may grow to become the large corpora calcificantia that may eventually acquire fibrous capsules, although Ross and associates were inclined to believe that the two forms of calcifications were distinct from each other.

Marine [5] described calcification in the adrenal glands of cats and believed it to be a lesion residual to an attack of feline distemper (it had not yet been proved at the time of Marine's observations that distemper is caused by a virus); he stated that distemper was followed, sometimes in two to three weeks, by a clinical syndrome similar to that developing after bilateral adrenalectomy. In man, adrenal calcification is well known in cases of adrenal hemorrhage or tissue necrosis (e. g., in tuberculosis) and has been described in rats deficient in pantothenic acid (Schultz, Winters, and Krehl,[11] 1952). However, the cytological alterations found in the cats of the present study have not been found in man or the rat.

The question arises: Are the changes described in the cat the result of a disease process, or simply the normal appearance of the adrenal glands of cats? The frequency of the structural alterations, particularly fatty metamorphosis, and the absence of demonstrable clinical signs seem to indicate that fatty cellular vacuolation and calcareous bodies occur normally in the adrenal cortex of the cat. On the other hand, Marine [5] (1926) stated that his cats had suffered from distemper. Perusal of publications by Bennett [2] and Nicander [6] does not reveal evidence of any adrenal cortical alterations resembling those described here. The adrenal glands of some cats necropsied in the Pathology Branch of the Army Chemical Center were normal in structure. It is not reasonable that calcification of the severity exhibited by our cats could be due to anything other than previous injury to the tissue. The cats under discussion were obtained from dealers in Pennsylvania, had received no vaccinations, and were without evidence of abnormal clinical history; but they had undoubtedly been exposed to a variety of infectious diseases. It can only be concluded that these changes are true lesions, the sequelae of systemic disease, or of a specific disease of the adrenal glands. It is not known whether cats with the described lesions respond to stressful situations in the normal manner.

Summary

In a study of calcification in the adrenal cortex of cats (obtained from dealers in Pennsylvania), calcareous deposits were found in the adrenal cortices of 10 of 27 adult cats and fatty metamorphosis in 23 of the 27. There was no demonstrable difference in incidence of the lesions between sexes, and cats weighing less than 1000 gm. were found to be free of both lesions. Calcification occurred in a matrix of acid mucopolysaccharide, is considered to be a morphogenetic sequel to fatty metamorphosis, and is believed to be the result of some previous tissue injury.

Dr. J. R. M. Innes gave encouragement and advice in this study; Dr. A. D. Bergner prepared many of the slides, and Mr. John Cuculis made the photomicrographs.

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Experimental Renal Infarction

II. Histochemical, Fatty, and Morphologic Changes

THOMAS L. GAVAN, M.D.
and
NATHAN KAUFMAN, M.D., Cleveland

Introduction

Numerous studies have been carried out concerning the sequential morphologic changes following experimentally produced infarcts.* In recent years increasing attention has been drawn to the histochemical aspects of various pathologic processes.[5] In a previous report the alterations in some of the oxidative enzymes in experimentally infarcted kidneys were discussed.[6] Subsequent studies reported here include the changes observed in the amount of histochemically demonstrated alkaline phosphatase and 5-nucleotidase in the tubular epithelium following renal artery ligation. These enzymes participate in dephosphorylation and reflect the functional state of the proximal convoluted tubules.[7] The development of fatty degeneration is correlated with the alteration in the enzymatic activity.

Materials and Methods

Thirty-six albino male rats of the Sprague-Dawley strain, each weighing approximately 225 gm., were used. Each animal was placed under light ether anesthesia and the perinephric fat dissected from the left kidney. The left renal artery was then exposed and ligated with linen suture. If additional or aberrant arteries were found, they too were ligated. Within 30 seconds the kidney became swollen and took on a bluish hue. The opposite renal artery was left intact so that the right kidney could serve as a control. The abdomen was then closed with linen suture. The animals were killed in groups of four at intervals of 1, 2, 4, 8, 12, 18, 24, 36, and 48 hours after ligation.

Tissue from each kidney was immediately placed in the following fixatives: 10% neutral formalin, cold acetone-alcohol mixture, and absolute alcohol. Paraffin sections were studied, using the following procedures: the hematoxylin and eosin stain, the periodic acid-Schiff (PAS) reaction, the diastase-PAS reaction, Best's carmine stain, the Feulgen reaction, and the calcium-cobalt methods for alkaline phosphatase and 5-nucleotidase. Frozen sections were also prepared and stained with Sudan IV.

The enzymatic activity, as well as the amount of PAS-positive material present, was graded from 0 to 4+ by comparison of the infarcted kidney with the undisturbed (right) kidney. In sections graded 4+ the enzymatic activity and amount of PAS-positive material were the same in both experimental and control kidneys. Sections graded 0 showed no detectable enzyme activity or PAS-positive material in the infarcted tissue.

The amount of neutral fat present was also graded 0 to 4+. Those sections graded 4+ showed the maximum amount of sudanophilic material in this series.

Results

5-Nucleotidase activity was confined to the luminal border of the proximal convoluted tubule (Fig. 1). No decrease in enzymatic activity was detected one hour after renal artery ligation. At two hours a slight decrease was seen. The activity of this enzyme then decreased steadily, so that after 48 hours no enzymatic activity was demonstrable except in a narrow subcapsular zone (Table; Fig. 2).

Alkaline phosphatase activity showed a distribution similar to that of 5-nucleotidase (Fig. 3); however, no decrease in enzymatic activity was evident before four hours had elapsed. At the end of a 48-hour period a trace of enzymatic activity was still present in occasional tubules, particularly in the inner cortical zone and subcapsular zone. There was no evidence of activity in the

Submitted for publication June 8, 1956.

From the Departments of Pathology, Cleveland City Hospital and Western Reserve University School of Medicine.

* References 1-4.

Average Enzymatic Activity and Content of PAS-Positive Material and Neutral Fat in Kidney Following Complete Renal Artery Ligation

Determination	Time, Hr.								
	1	2	4	8	12	18	24	36	48
5-Nucleotidase	++++	+++	+++	+++	++	++	+	Trace	0
Alkaline phosphatase	++++	++++	+++	++	++	+	+	+	Trace
PAS	++++	+++	+++	+++	++	++	++	++	+
Fat	0	0	0	0	+	++	+++	++++	++++

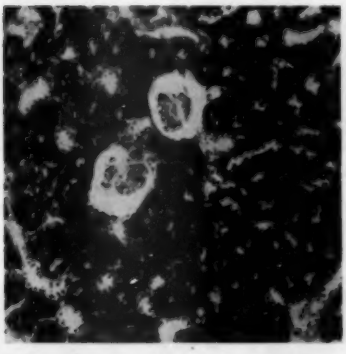

Fig. 1.—Normal distribution of 5-nucleotidase activity in renal tubules. × 100.

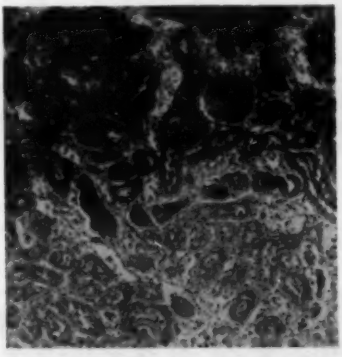

Fig. 2.—5-Nucleotidase activity 48 hours after renal artery ligation. Enzymatic activity is absent except in subcapsular zone in upper part of illustration. × 100.

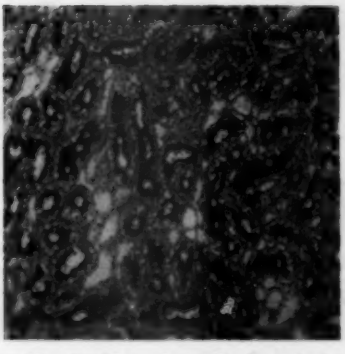

Fig. 3.—Normal distribution of alkaline phosphatase activity in renal tubules. ×100.

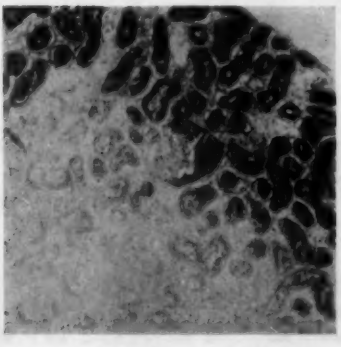

Fig. 4.—Alkaline phosphatase activity 48 hours after renal artery ligation. Enzymatic activity is absent except in subcapsular zone in upper part of illustration. × 100.

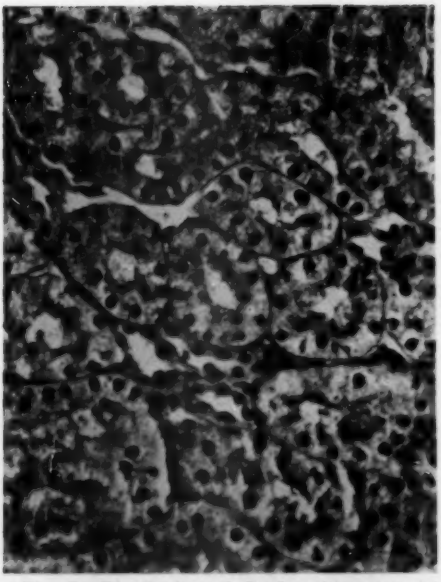

Fig. 5.—Distribution of PAS-positive material in control kidney. Note the numerous fine PAS-positive granules in the luminal aspect of the proximal convoluted tubule epithelium. Reduced to 90% of mag. × 360.

Fig. 6.—PAS-stained section of kidney 48 hours after renal artery ligation. This illustrates the marked loss of PAS-positive material. Reduced to 90% of mag. × 360.

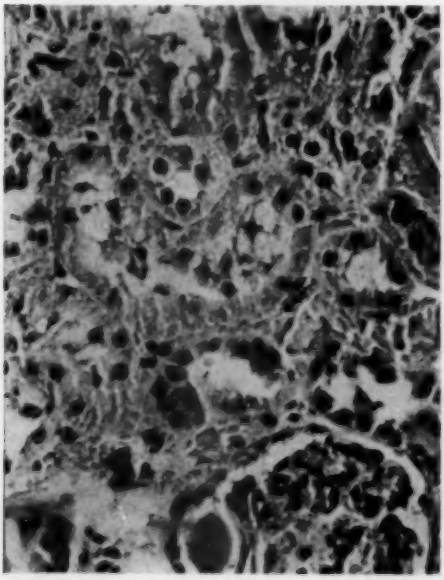

Fig. 7.—Left kidney one hour after renal artery ligation, showing early tubular degenerative changes. Hematoxylin-eosin stain; reduced to 90% of mag. × 360.

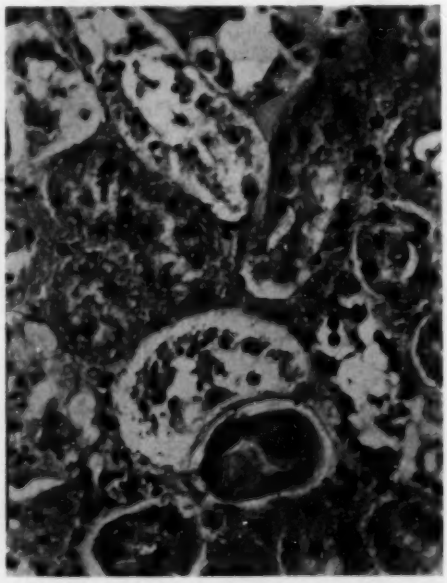

Fig. 8.—Left kidney four hours after renal artery ligation, showing varying degrees of degeneration and necrosis of all tubular elements. Hematoxylin-eosin stain; reduced to 90% of mag. × 360.

remainder of the renal tubules (Table; Fig. 4).

Sections stained by the periodic acid-Schiff reaction revealed a PAS-positive, diastase-fast material in the luminal portion of the proximal convoluted tubular epithelium. Best's carmine stain for glycogen was negative. The general distribution of this substance corresponded with that of alkaline phosphatase and 5-nucleotidase (Fig. 5). Two hours after renal artery ligation there was a slight decrease in the amount of this tity remained in some tubules, while in many substance. After 48 hours a moderate quantubules with completely necrotic epithelium it was absent (Table; Fig. 6).

Neutral fat was not evident until 12 hours following ligation. At this time an occasional proximal convoluted tubule contained fine intraepithelial droplets of sudanophilic material. The maximum amount of fat was seen in infarcts 36 and 48 hours old (Table).

Nuclear degeneration, in Feulgen-stained sections, became evident four hours after ligation. The usual picture of nuclear death, pyknosis, and karyorrhexis was especially well demonstrated by this method.

In hematoxylin-eosin sections morphologic changes were seen one hour after ligation. Hyperemia at the corticomedullary junction was marked, and evidence of cloudy swelling and slight hydropic degeneration was present. Necrosis was seen in the occasional cell (Fig. 7). These degenerative changes rapidly progressed, so that necrosis of all tubular elements was present after four hours (Fig. 8). Tubules located in the inner cortical zone were in a better state of preservation than those in the remainder of the cortex. In tubules in immediate contact with the renal capsule, those epithelial cells on the most peripheral border were well preserved, while the remaining cells were necrotic.

Comment

When the histochemical and morphologic changes were compared, it was noted that, although cytoplasmic changes were evident in the first hour, no histochemical changes could be demonstrated at that time.

Histochemical changes were first seen at two hours, with a slight decrease in both 5-nucleotidase activity and PAS-positive material. This was followed at four hours by a decrease in alkaline phosphatase activity. In a previous experiment, succinic dehydrogenase and cytochrome oxidase activity was studied in experimentally infarcted kidneys. The initial decrease in activity of these enzymes also occurred after four hours of ischemia.[6] This sequence is similar to that seen in the dog myocardium by Kent and Diseker,[8] who found that morphologic changes became evident 9 hours after coronary artery ligation but that a decrease in succinic dehydrogenase activity did not occur until 15 hours had elapsed.

The retention of enzymatic activity in the subcapsular zone is thought to be due to the presence of some blood supply through the capsular vessels. Although enzymatic activity appears to be sufficient, it may be decreased to an extent which cannot be appreciated by our methods. A similar persistent zone was noted by Goebel and Puchtler in their studies of the effect of renal artery ligation on the alkaline phosphatase and 5-nucleotidase activity.[9]

The nature of the PAS-positive material cannot be completely evaluated at present. This material is not glycogen, since it is resistant to diastase digestion and, in addition, Best's carmine stain is negative for glycogen. It is unlikely that this material is an acid mucopolysaccharide, since it gives a nonmetachromatic reaction with toluidine blue.

Alkaline phosphatase and 5-nucleotidase both act as important participants in dephosphorylation reactions. In the kidney it has been shown that alkaline phosphatase activity is related to its functional state.[7] Specifically, alkaline phosphatase plays an important role in glucose reabsorption. Its action here is the dephosphorylation of hexose phosphates, with the release of glucose. The specific enzyme in this reaction is most

probably glucose-6-phosphatase.[10] 5-Nucleotidase is responsible for the hydrolysis of adenylic acid, forming adenine + ribose-phosphoric acid. This reaction is related to purine metabolism and may form an important step in the elimination of purine metabolites by the renal tubules.

Fatty degeneration of the tubular epithelium was not observed until enzymatic activity had decreased markedly. It would seem that fatty degeneration is inversely related to the level of enzymatic activity, since with progressive decrease in enzymatic activity there was a concomitant increase in the amount of fat seen. Furthermore, McManus[11] has proposed that fatty degeneration in renal tubules is due to a disturbance in phospholipid metabolism. He believes that the basic fault lies in the failure of phosphate binding to the triglyceride and/or the choline or ethanolamine combination. A decrease in available phosphate, preventing phosphorylation, is a possible mechanism that may interfere with phosphate binding.

We have shown that alkaline phosphatase and 5-nucleotidase are markedly decreased before fatty degeneration is observed. It is conceivable that, because of this decreased enzymatic activity, phosphate, which would normally be derived from dephosphorylation of hexose phosphates and nucleotides, is not available in sufficient quantities for adequate phosphorylation of phospholipid bases.

Summary

The sequential changes in alkaline phosphatase, 5-nucleotidase, PAS-positive material, and neutral fat have been studied in experimentally infarcted kidneys. Alkaline phosphatase and 5-nucleotidase activity showed a decrease only after definite histologic evidence of cellular damage was present. The presence of a PAS-positive, diastase-fast material was noted in the epithelium of the proximal convoluted tubules. This substance decreased in amount following renal artery ligation. It was observed that the fat content of the tubular epithelium increased as enzymatic activity decreased.

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Occlusion of a Coronary Artery

A Study of One Hundred Ninety-Five Cases

BÉLA HALPERT, M.D.
ETHEL E. ERICKSON, M.D.
and
FERENC GYORKEY, M.D., Houston, Texas

The observations at necropsy are herein recorded on patients who died of occlusion of a coronary artery in the Veterans Administration Hospital, Houston, Texas. This study includes the incidence, the site and nature of the lesion, contributory causes, and duration of the illness.

Material and Methods

Between Oct. 1, 1949, and Oct. 24, 1955, a total of 1800 male patients were examined after death, comprising about 90% of those who died in the hospital. Of these, occlusion of a coronary artery with infarction or extensive scarring of the myocardium was observed in 195.

Most of the hearts were fixed in formalin (10%) prior to sectioning. Parallel cuts perpendicular to the axis of the heart were then made, and the specimen was immersed in alcohol (80%) to restore its color. By this method the location and extent of the infarcted or scarred area and the approximate site of occlusion of the coronary artery supplying the area could be determined. In most instances photographs were made of the hearts, many of them in color. In addition to the routine microscopic preparations, paraffin or celloidin sections were secured for this study from new blocks of tissue obtained from the coronary artery and from the area of infarction. These sections were stained with hematoxylin and eosin and by Masson's trichrome method. An additional section from the coronary artery was stained with Weigert's elastic tissue stain. All other organs, particularly the kidneys and brain, were reviewed.

Incidence

Necrospsies were performed on 1344 white and 456 Negro patients. Occlusion of a coronary artery was observed in 165 of the white and in 30 of the Negro subjects. The age distribution of all patients examined after death and of those with coronary occlusion is given in Table 1. The youngest patient with coronary occlusion was 32 and the oldest 83 years of age.

Site of the Lesion

The left ventricle was involved in 177, the right ventricle or atrium in 6, and both ventricles in 12 instances (Table 2). According to location in each ventricle, the lesions were anteroseptal, lateral, and posteroseptal, et cetera. Among the 101 patients with anteroseptal infarction or scarring of the left ventricle, 9 had aneurysmal dilatation of the apex and 2 additionally had rupture and hemopericardium.

Nature of Lesions

Changes in the Myocardium.—The gross changes in the myocardium were similar, irrespective of the location of the lesion (Figs. 1, 3, 5, 7, and 9). In most instances they consisted of recent infarction of the previously uninvolved myocardium, usually affecting most of the thickness of the wall. In some instances the infarction was superimposed on an area of extensive scarring of the myocardium or there was extensive scarring with minimal evidence of recent infarction. The changes in the myocardium varied according to the age of the infarct and the condition of the myocardium prior to the event.[1]

In any recently infarcted area there was marked hemorrhage and necrosis with considerable thinning of the wall (Fig. 7). Streaks or white lines of previous scarring could be made out in the adjacent, less in-

Submitted for publication June 7, 1956.

From the Department of Pathology, Baylor University College of Medicine, and the Veterans Administration Hospital.

TABLE 1.—*Age Incidence of Occlusion of a Coronary Artery Among 1800 Patients Examined After Death*

Age, Yr.	Patients	Occlusion of a Coronary Artery	Percent
Unknown	4 (1 M W, 3 M N)	0	--
Under 30	139 (108 M W, 31 M N)	0	--
31-40	233 (173 M W, 60 M N)	13 (10 M W, 3 M N)	6 5
41-50	205 (153 M W, 52 M N)	26 (23 M W, 3 M N)	15 6
51-60	604 (452 M W, 152 M N)	75 (60 M W, 15 M N)	13 10
61-70	500 (348 M W, 152 M N)	69 (60 M W, 9 M N)	17 6
71 and over	115 (109 M W, 6 M N)	12 (12 M W, 0 M N)	11 0
Total	1800 (1344 M W, 456 M N)	195 (165 M W, 30 M N)	12 6

volved areas. In contrast to the involved area, the uninvolved myocardium was usually increased in thickness. In some instances mural thrombi were attached to the endocardial surface (Fig. 5). Microscopically, in an area of recent infarction there was extensive destruction of myocardial fibers. The fibers remaining were spread apart, and their striations were faded (Fig. 10). There was infiltration of erythrocytes, granulocytes, and some lymphocytes, plasma cells, and large mononuclear cells in the connective tissue stroma.

TABLE 2.—*Site of the Lesion*

Left ventricle		177
Anteroseptal infarction	101	
Lateral infarction	39	
Posteroseptal infarction	19	
Antero- and posteroseptal infarction	7	
Entire circumference infarcted	6	
Septal infarction	2	
No demonstrable infarction	3	
Right ventricle		6
Anteroseptal infarction	2	
Lateral infarction	1	
Atrial infarction	2	
No demonstrable infarction	1	
Both ventricles		12

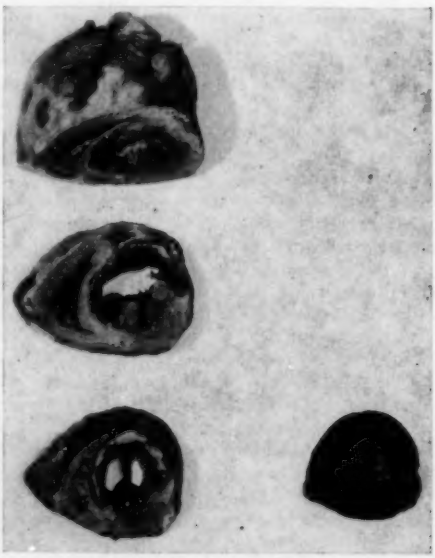

Fig. 1.—Infarction of myocardium, anteroseptal, in a white man aged 38. The heart measured 12 cm. from base to apex and 10 cm. across the base, and weighed 400 gm.

In a healed infarcted area there was re-

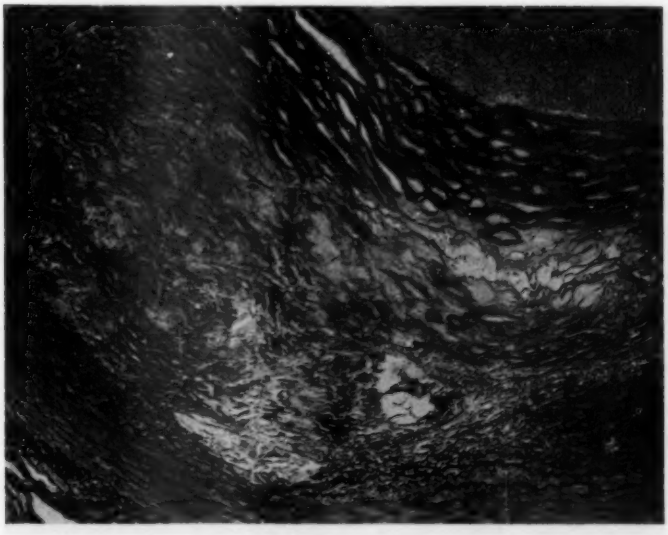

Fig. 2. — Microscopic section of heart shown in Figure 1. Atheromatous and sclerotic changes in the intima and media narrow the lumen of the anterior descending branch of the left coronary artery. There is reduplication of the inner elastic lamina. Weigert's resorcin-fuchsin elastica stain; reduced to 80% of mag. × 100.

Fig. 3.—Infarction of myocardium, anteroseptal, in a Negro aged 37. The heart measured 14 cm. from base to apex and 12 cm. across the base, and weighed 500 gm.

placement of myocardial fibers by dense hyalinized fibrous connective tissue. The scarred areas usually blended with the less involved or uninvolved thick myocardium. Microscopically, in such areas the myocardial fibers varied in width and were encased or spread apart by hyalinizing or densely hyalinized fibrous connective tissue.

Changes in the Coronary Arteries.— Atheromatous, sclerotic, and calcific changes in the intima and media narrowed the lumen of the coronary artery or its branches (Figs. 2, 4, 6, and 8).* In some instances the lumen was narrowed to a pinpoint or occluded by an organizing or recent thrombus. The microscopic appearance varied accordingly. The eccentric thickening of the wall by atheromatous, sclerotic, and calcific changes in the intima and media disrupted the inner elastic lamina. Reduplication of the lamina nearby was noted (Fig. 2).

When the lumen was obliterated by loose connective tissue, dilated endothelium-lined spaces recanalized the site of previous occlusion. Occasionally these spaces were filled with recent thrombi (Fig. 4). When a thrombus occluded the lumen, the sclerotic and atheromatous change in the media and intima usually blended with the thrombus (Fig. 6). In other instances sclerotic and calcific changes distorted the intima and media and the lumen was occluded by loose

* References 2, 3.

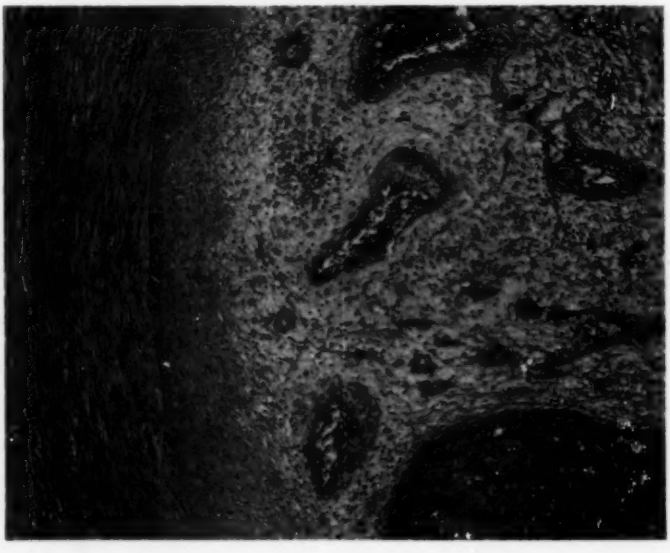

Fig. 4. — Microscopic section of heart in Figure 3. The lumen of the anterior descending branch of the left coronary artery is obliterated by loose connective tissue containing many endothelium-lined spaces (recanalization), one filled with a recent thrombus. Masson's trichrome stain; reduced to 80% of mag. × 100.

Fig. 5.—Infarction of myocardium, anteroseptal, with aneurysm and mural thrombus, in a white man aged 56. The heart measured 13 cm. from base to apex and 15 cm. across the base, and weighed 980 gm.

connective tissue, containing scattered large mononuclear cells with brown granules in their cytoplasm (Fig. 8). A lumen narrowed by atherosclerotic change became further narrowed or occluded by a recent hemorrhage into the atheromatous area (Fig. 6).†

† References 4, 5.

Contributory Causes

In the course of this study the following contributory causes were considered: nephrosclerosis and cardiac hypertrophy accompanying hypertension; obesity, and the nature of the patient's occupation.

Some degree of nephrosclerosis was observed microscopically in every instance.

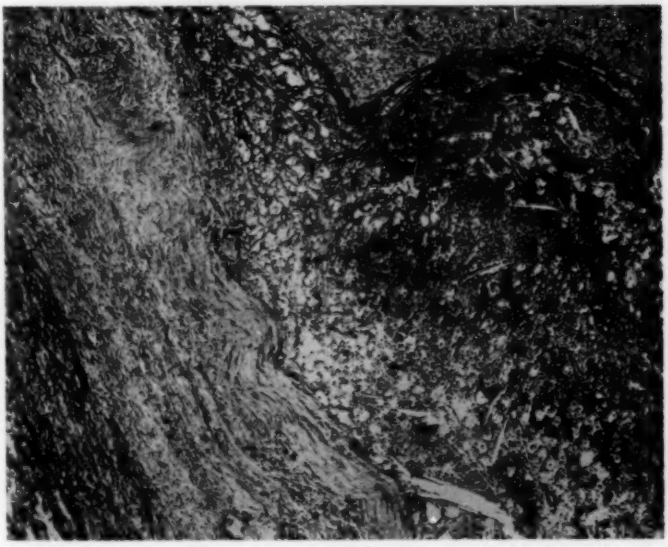

Fig. 6. — Microscopic section of heart in Figure 5. A recent thrombus occludes the lumen of the anterior descending branch of the left coronary artery. Marked atheromatous change in the intima and media narrow the lumen. There are many erythrocytes, slit-like spaces, and large mononuclear cells, with foamy cytoplasm. Masson's trichrome stain; reduced to 80% of mag. × 100.

Fig. 7.—Infarction of myocardium, lateral, left, in a white man aged 45. The heart measured 14 cm. from base to apex and 14 cm. across the base, and weighed 550 gm.

The combined weight of the kidneys was less than 400 gm. in 154 of the 195 patients. In practically every instance was observed some degree of variation in size of the myocardial fibers, with bizarre-shaped, deeply stained nuclei, considered as microscopic evidence of hypertrophy. The heart weighed less than 350 gm. in 17 patients; up to 450 gm. in 44, to 550 gm. in 66, and to 650 gm. in 34; and over 650 gm. in 34 patients. Nephrosclerosis with cardiac hypertrophy, presumed evidence of hypertension in the absence of a valvular lesion, was recorded in 178, i. e., in over 90% of the patients.

Clinical evidence of hypertension (over 140 mm. Hg systolic and over 90 mm. diastolic) was actually recorded in 144 patients. Of the remaining 51, the blood pressure was normal or subnormal in 43, and 8 were dead on arrival at the hospital.

Myocardial damage was recorded by electrocardiographic examination in 183 of the 195 patients.

Obesity (over 180 lb., or 81.6 kg.) was recorded in 73 of the 195 patients. Clinical evidence of diabetes mellitus was present in 14 patients.

The occupation of 129 of the 195 patients was given as follows: skilled laborer, 59; professional, 40; unskilled laborer, 22; farmer, 8.

Duration of Illness

"Heart attack" in 97 patients, without previous signs or symptoms, led to death immediately or within one week to three months. Death of 21 patients occurred after three months to one year.

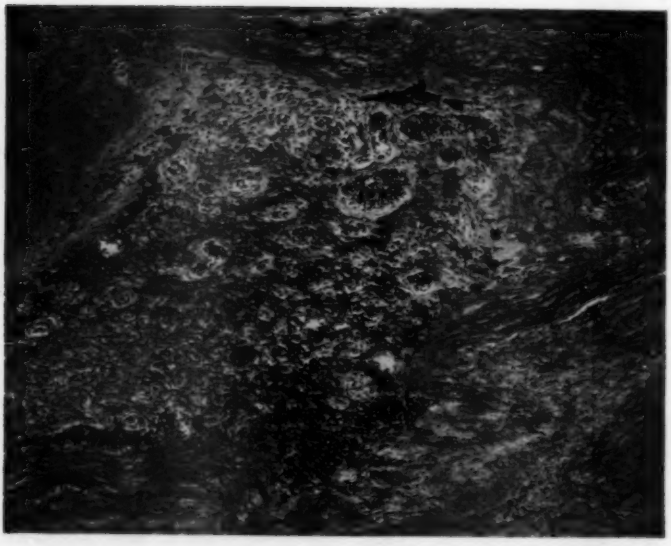

Fig. 8. — Microscopic section of heart in Figure 7. The lumen of the anterior descending branch of the left coronary artery is filled with connective tissue containing many blood vessels. There is distortion of the vessel wall by sclerotic change. Masson's trichrome stain; reduced to 80% of mag. × 100.

Fig. 9.—Infarction of the entire circumference of the left ventricle in a white man aged 54. The heart measured 15 cm. from base to apex and 16 cm. across the base, and weighed 600 gm.

Clinical evidence of one previous attack was available in 42 patients, and of two previous attacks in 13 patients, the interval between attacks usually diminishing. In the remaining 22 patients clinical record of myocardial damage of from 3 to 20 years existed. In these patients there was gross and microscopic evidence of infarction of the myocardium, old or recent (Table 3).

TABLE 3.—*Duration of Illness*

Died of first attack		97
Immediately or within one week	45	
During 2d week	17	
During 3d to 12th week	35	
Survived first attack 3 mo. to 1 yr.		21
Survived one previous attack		42
Over 4 yr., 4; four years, 5; three years, 5; two years, 14; one year, 9; half-year, 5		
Survived 2 previous attacks		13
5 to 15 yr. between first and second, and 2 to 3 yr. between second and third attacks	5	
1 to 4 yr. between first and second, and ½ to 2 between second and third attacks	8	
All died 1 day to 5 mo. following third attack		
Lived from 3 to 20 yr.		22
Clinical record of myocardial damage and gross and microscopic evidence of old or recent infarction of myocardium		

Incidental Observations

Atherosclerotic changes in the cerebral arteries, with varying degrees of encephalomalacia of the hemispheres, occurred in 46 of the patients. Aneurysm of the abdominal aorta was recorded in 12. Thrombosis in the

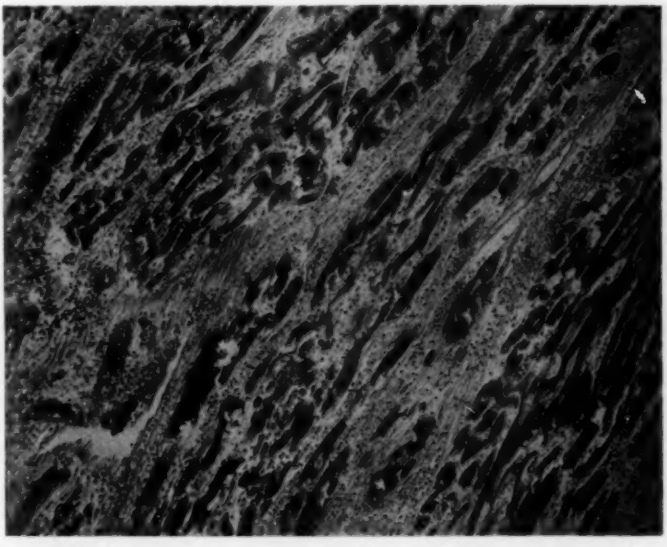

Fig. 10. — Microscopic section of heart in Figure 9. The myocardial fibers are spread apart; their striations are faded. There is an infiltration with erythrocytes, granulocytes, lymphocytes, plasma cells, and large mononuclear cells in the connective tissue stroma. Masson's trichrome stain; reduced to 80% of mag. × 100.

abdominal aorta and in some of its branches occurred in 16 patients: The aorta was involved in six; the iliac arteries, in six (necessitating amputation in three), and the superior mesenteric artery, in four. Syphilitic valvulitis and aortitis were recorded in six instances. Polyarteritis nodosa was diagnosed in two cases. Infarcts in the lungs were observed in 21 patients, in the spleen in 8, in the kidneys in 7, in the hypophysis in 2, and in the liver in 1.

Marked fatty change of the liver was recorded in 17 patients and portal cirrhosis in 8; chronic cholecystitis with cholelithiasis was present in 13; chronic cholecystitis without cholelithiasis in 4, and cholelithiasis without cholecystitis in 1 of the patients.

Chronic ulcer of the stomach occurred in five and of the duodenum in four.

Carcinoma was observed in 13 patients: of the lung in 4, the prostate in 3, the stomach in 2, and the pancreas, rectum, kidney, and urinary bladder in 1 each.

Comment

The incidence of myocardial infarction observed at necropsy has been extensively investigated.‡ The high incidence in our series is attributable to the circumstance that all the patients were men. Our study revealed a fairly accurate correlation among clinical data, electrocardiographic recordings, and necropsy findings. The observations are in accord with other studies on distribution of the coronary arteries, sites of occlusion, and collateral circulation.§ Whatever the predisposing or inciting causes of atherosclerosis responsible for the occlusion of a coronary artery may be,[12] hypertension, when present, is an obvious contributing factor. The occlusion, our studies reveal, is usually preceded by a period of transient, subclinical, or clinical hypertension. This relationship between hypertension and coronary occlusion has recently been reinvestigated, and the conclusions seem to be in line with our own impressions.[13] Excess strain on the myocardium because of increased work demand and inadequate blood supply is apparently the cause of the myocardial infarction in the earlier decades of life.‖ In the later years clinical hypertension is usually the cause. Though not all patients with hypertension develop coronary occlusion and infarction of the myocardium, the dynamics of their circulation predispose them to such lesions. In such patients the

‡ References 6, 7.

§ References 8-11.

‖ References 14-16.

hypertension further injures the renal vessels—arteries, arterioles, and glomeruli—thus increasing the hypertension and the atherosclerosis everywhere, including the arteries supplying the brain and heart. The importance of strain on the myocardium as a contributing factor is further supported by the observation that the occlusion usually occurs in the left coronary artery, the vessel supplying the harder-working, left ventricle.

It should be emphasized that infarction of the myocardium occurs only when the occlusion is sudden and involves a substantial branch of a coronary artery. A gradual narrowing of the lumen of a coronary artery or any of its branches results in an increase of the connective tissue stroma, with eventually diffuse scarring of the myocardium.

Summary

Among 1800 male veteran patients, 1344 white men and 456 Negroes, occlusion of a coronary artery with infarction or extensive scarring of the myocardium occurred in 165 of the white and 30 of the Negro subjects. The lesion involved the left ventricle in 177, the right ventricle in 6, and both ventricles in 12 instances. Of the contributory causes considered, nephrosclerosis was encountered in all patients, cardiac hypertrophy in 178, with the heart weighing over 450 gm. in 134, and obesity in 73. Of the 195 patients, 22 lived from 3 to 20 years with clinical evidence of myocardial damage and gross or microscopic evidence of old or recent infarction of the myocardium; 13 survived two previous attacks, and 42, one previous attack. Of the remaining patients, 21 survived the first attack from three months to one year, and 97 died of the first attack immediately or within three months.

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Melanuric Nephrosis

J. C. ROSENBERG, M.D., Minneapolis

Introduction

The accelerated formation of melanin and its intermediates which takes place in malignant melanomas occasionally results in complications of a most unique character. The following case report demonstrates one of the more uncommon of these occurrences. It is presented for the interest it may have and the possible insight it may afford into the biology of the malignant melanoma and the pigment it produces.

Report of a Case

Clinical Course*

Present Illness.

A 53-year-old white business man was referred to the Second Medical Clinic of the Allgemeines Krankenhaus, Vienna, on Dec. 31, 1955, with a diagnosis of malignant melanoma with diffuse metastases. His past history revealed that in 1924 a neuroma was extirpated from his right cubital fossa. In 1925 a second operation was necessary, and again a neuroma was removed. In 1947 he observed a nevus on the upper third of his right forearm. In October, 1954, the patient had this pigmented nevus removed because it had become ulcerated. Histologic examination revealed it to be a malignant melanoma. In July, 1955, the right axillary lymph nodes were the size of plums. They were removed and the area injected with radioactive gold. For the four to five weeks prior to admission the patient became progressively weaker, complained of abdominal pain, and showed a moderate degree of abdominal distention.

Family History.

The patient's grandmother died of a cancer of the uterus; her sister died of a cancer of the breast. Of her four brothers, one died as a result of a cancer of the stomach, and another died of a tumor of the urinary bladder. The other two died of "old age." However, one of the latter had a son who died at 61 years of age, as a result of a malignant melanoma. One of the four siblings of the patient's mother died of Hodgkin's disease. The patient's mother was living and well, but his father had died of a cancer of the urinary bladder. One of the patient's nephews died at 11 years of age as a result of a malignant melanoma.

Physical Examination.

The positive findings were as follows: Upon examination of the lungs, bilateral posterior rhonchi were heard. The abdomen was distended. A hard, irregular liver edge could be felt 4 cm. below the right costal margin in the midclavicular line. The right axillary lymph nodes were tender, hard, and the size of dates. The skin of this area contained a scar 10 cm. in length. In the upper third of the right forearm a barely visible scar was noted. Both these scars were surrounded by several hard, dark-bluish nodules.

Hospital Course.

On Jan. 7 icterus developed and became progressively worse. On Jan. 8 the patient exhibited oliguria and signs of renal failure and uremia. The latter became progressively worse, until Jan. 17, 1956, when he quietly passed away.

Autopsy Findings

Gross Examination.

At necropsy, the external findings confirmed the clinical observations mentioned above. The pleural spaces were free. Numerous subpleural, darkly pigmented, sharply demarcated, cherry-sized nodules were diffusely distributed in the parenchyma of the lungs. In the posterior wall of the left ventricle, a pea-sized nodule of the above type was noted. The thyroid, paratracheal, and hilar lymph nodes also contained these darkly pigmented nodules. The peritoneal cavity contained about 2 liters of clear yellow fluid. The liver was enlarged to about twice its normal size and contained numerous cherry-sized, darkly pigmented nodules. The parenchyma was largely replaced by this tissue and was small in amount and greenish-yellow in color. The omentum, peritoneum, spleen, adrenals, and a section of the spinal column also demonstrated the above-described nodules. The kidneys were of the usual size. Their capsules stripped with ease, revealing

Submitted for publication April 10, 1956.

Formerly Fulbright Fellow in Pathology; at present, Fellow in Surgery at the University of Minnesota Hospitals.

From the Institute of Pathologic Anatomy of the University of Vienna (Director: Prof. H. Chiari) and the Vienna Allgemeines Krankenhaus.

* Clinical material was obtained through the courtesy of Prof. K. Fellinger, Head of the University of Vienna Second Medical Clinic of the Allgemeines Krankenhaus.

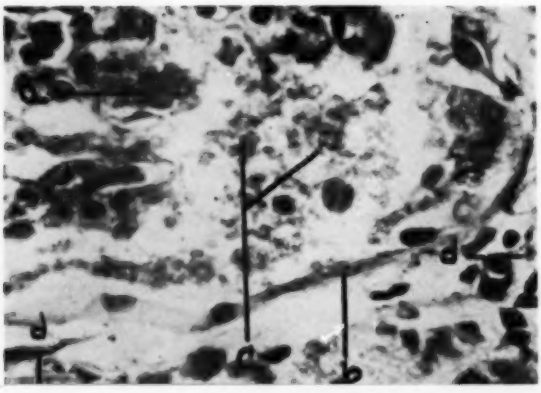

Fig. 1.—Preparation of the kidney using Masson's silver impregnation technique. The melanin pigment granules can be seen in the cells of the glomerulus (*a*), in Bowman's capsule (*b*) and space (*c*), and in the interstitial tissue (*d*).

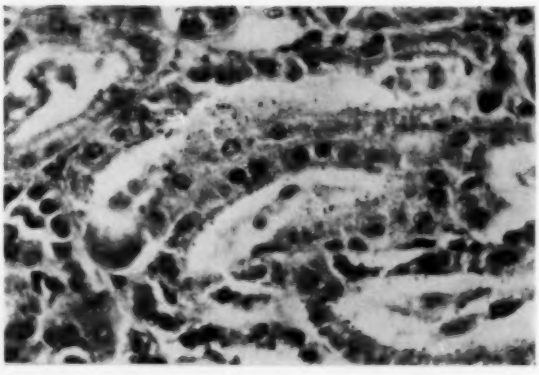

Fig. 2.—Preparation of the kidney using Masson's silver impregnation technique. The tubular epithelium contains a fine granular deposit of melanin at the superficial margin of the cells.

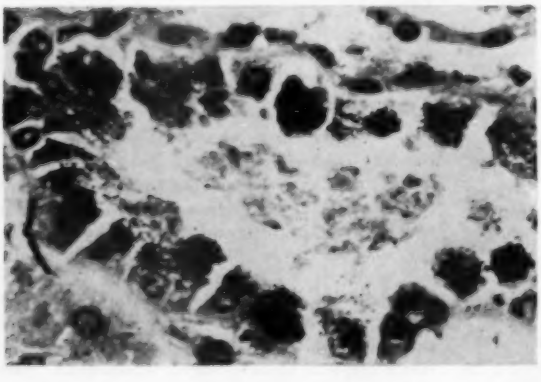

Fig. 3.—Preparation of the kidney using Masson's silver impregnation technique. The tubular cells pictured here are largely filled with melanin pigment.

a diffuse brown-black color of the surface, which was otherwise smooth and unremarkable. On cut section, the cortex also appeared brown-black, the medulla being slightly darker. Clearly visible darker brown-black stripes extended from the cortex to the medulla. Except for a uremic gastroenterocolitis, the remainder of the gross findings were unremarkable.

Microscopic Examination.

Sections of the kidneys, stained with hematoxylin and eosin, showed the glomeruli to be of the usual cellularity. Bowman's spaces were frequently filled with granular detritus and small brown-black granules.

The latter gave a negative histochemical reaction for iron but reacted positively with Masson's silver impregnation technique (Fig. 1*c*). In some areas, fine, similarly colored granules could be distinguished within the epithelium of the glomerulus (Fig. 1*a*). Occasionally they could be found in the interstitial tissue (Fig. 1*d*). By using Masson's silver impregnation technique, all of this granular material was stained black. The tubular cells exhibited cloudy swelling. In the periphery of some of the tubular cells a fine granular deposit could be seen (Fig. 2). The cytoplasm of other cells was nearly completely filled with these pigmented granules (Fig. 3). The latter condition was most commonly seen in the cells of the proximal convoluted tubules of the corticomedullary areas. The lumina of the loops of Henle, especially the excretory ducts, were often filled with casts having a brown-black color and giving an intensely positive Masson reaction. The epithelium cells here were free of this pigment. Staining with Sudan III for fat, in frozen sections of the kidney, gave negative results. No evidence of an inflammatory reaction was present. However, in several areas fine brown-black granules could be seen in the cells of the interstitial tissue. This granular material gave the same positive reaction with Masson's silver impregnation technique (Fig. 1*d*). In one area, a blood vessel was seen to contain a group of cells which had the typical appearance of a malignant melanoma.

Diagnoses.

The final diagnoses were as follows:

1. Multiple malignant melanomas, metastatic from a primary malignant melanoma of the skin of the upper right arm (operated on 15 months previously) to the right axillary, paratracheal, and hilar lymph nodes, and the lungs, heart, thyroid, liver, spleen, adrenals, and spinal column.
2. Melanuric nephrosis.
3. Uremic gastroenterocolitis.

Comment

A recent comprehensive review of the literature on melanomas cited the work of Fitzpatrick, Lerner, Becker, and Montgomery as being "productive of important fundamental findings with regard to histogenesis" of this tumor and the histochemistry of melanogenesis.[1] These workers have recently written on another rare complication of melanomas which arises from the excessive production of pigment, i. e., the transformation of the normal color of the skin.[2] As a result of their extensive investigations and experiences, they have been able to put forth an excellent explanation of the pathogenesis of such complications. The melanoma cell, producing increased amounts of melanin from tyrosine, liberates certain intermediates, e. g., dopa and 5,6-dihydroxyindole. These substances are taken up by the blood and may cross the capillary membrane in any part of the body. The conversion to melanin (by oxidizing systems other than the tyrosinase system) can take place within histiocytes or the extracellular fluids.

In the case reported here, the organ where this pigment was accumulated was the kidney and the lesion it produced was a melanuric nephrosis. The paucity of material in the literature on this subject attests to its rarity. However, nephropathy produced by the accumulation of pigments in the kidney is not uncommon.

The most recent mention of pathologic findings similar to those which we encountered was by Gössner. He described a case of "pigment-storage nephrosis" (*Pigmentspeicherungsnephrose*) due to a lipochrome (lipofuscin).[3] A pigment nephropathy due to lipochromes, however, has been previously observed,[4] and studies of lipochrome deposits in the kidney have been made.† Similar studies concerning melanin deposition in the kidney are not as numerous, despite the fact that the problem of melanuria has been actively considered since Zeller first described a means of detecting

† References 5-7.

melanuria, in 1883.[8] The best descriptions of melanin we could find were those by Jacobsen and Klinck[9] and by Lubarsch.[10] The former, using silver staining techniques, "studied the distribution of melanin in normal kidneys of adult Negroes, in patients with Addison's disease, in Negroes with malignant melanomas with generalized metastases, in blond and brunette white persons and in more than four hundred white mice, each of which had a melanoma derived from one originally discovered by Harding and Passay." These authors found that in their material the kidneys contained much pigment in the peripheral portions of the epithelium of the renal tubules. These tubules were also often seen to contain melanin pigment casts. Lubarsch's description of his experiences with melanin deposition in the kidney is somewhat more detailed. He noted that the kidneys not involved with neoplastic tissue were the ones that usually demonstrated melanotic deposits, an observation with which our case conforms. Grossly, the findings were characteristic. The kidney was gray-black to dark-brown-black, the latter color also being present as stippling and/or stripes extending from the cortex to the medulla. Histologically, the pigment was usually located in the proximal convoluted tubules and less often, in order of frequency, in the loops of Henle, the distal convoluted tubules, and the collecting tubules. He also noted the presence of melanin pigment casts. Rarely was there melanin in the epithelium of the glomerulus. He makes no mention of the pigment being found in the interstitial tissue. Melanin in these last sites is nicely demonstrated in the present case (Fig. 1*a* and *d*).

Cawley's[11] scholarly presentation of the genetic factors in malignant melanomas prompts us to comment briefly on the family history of our patient. It was the third malignant melanoma which occurred in a family with a high incidence of malignant tumors. The impossibility of making a statistical analysis of the situation permits us only to mention this finding, as was done by Fitzpatrick, Steves, and Scholtz[11] and by Greifelt.[12]

Summary

The occurrence of a severe melanuric nephrosis is described, and the literature on this subject is discussed. The concept that melanuric nephrosis is a form of "storage nephrosis" is supported by the findings reported herein and the recent elucidation of the histochemistry of melanogenesis.

The incidental finding of three cases of malignant melanoma in a family is mentioned.

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Late Lesions in Man Caused by Colloidal Thorium Dioxide (Thorotrast)

A New Case of Sarcoma of the Liver Twenty-Two Years After the Injection

JORGE da SILVA HORTA, Lisbon, Portugal

Thorotrast (colloidal thorium dioxide) was largely used in Portugal as contrast medium for x-rays as a result of the interest in the methods of vascular investigation. In 1925 Egas Moniz * attempted the visualization of the cerebral vessels with the aim of localizing cerebral tumors.

The first series of experiments were made on dogs; later experiments were carried out on human corpses and, finally, on volunteers. The contrast employed was strontium bromide. After the preparation of Thorotrast by Heyden Producers, the first results of hepatosplenography were presented by Dadt in 1930, using this substance, and later Egas Moniz employed Thorotrast also in cerebral angiography.

On the basis of the results of Egas Moniz, dos Santos and his colleagues [3] began using the same substance in arteriography of the limbs and in aortography. Thorotrast was also employed by other authors in Portugal and in other European countries for hepatosplenography, retrograde pyelography, mammography, cerebral ventriculography, and arthrography, but no doubt this drug was most used in vascular visualization. In Portugal, however, the drug has long ceased to be employed.

The wide use of this substance in Portugal explains the vast material which exists in the Department of Pathological Anatomy of the Faculty of Medicine of Lisbon. Studies on this material were first undertaken by my teacher, Prof. F. Wohlwill,† and later continued by me.‡

The investigation made in this department has dealt with two aspects: the distribution of the substance in the human body as time goes on and the lesions caused by the drug remaining in the body.

Recently my colleagues and I have been interested, like other authors, in the late effects of the drug. These late effects cause morbid states, which are now well defined as Thorotrast granulomas, panmyelopathies, liver cirrhosis (dystrophia lenta—Albertini), and neoplasms. Of the granulomas the most important are the paravascular ones. They are formed around the vessels which have been injected with the substance. Our experience is mostly connected with those of the neck. Their presence in this situation produces compressions on the important anatomical structures—the carotids, jugulars, trachea, esophagus, and nerves, the last sometimes being lost in the mass of the granuloma (brachial plexus; cervical sympathetic and recurrent nerves). The related symptoms are difficulty in movement of the head, pain in the neck and upper limbs, and dysphagia. The dysphonia caused by these granulomas (paralysis of the recurrent nerves) is well known to ear, nose, and throat specialists. One case of bilateral granuloma led to such severe asphyxia that an urgent tracheotomy was needed (Silva Horta [9]). The granulomas of the neck (encephalography) are the ones that cause most serious consequences.

Submitted for publication June 12, 1956.

From the Department of Pathological Anatomy, Faculty of Medicine; Director, Prof. J. da Silva Horta.

* References 1-2.

† References 4-6.

‡ References 7-9.

The granulomas of the hilus of the kidney due to retrograde pyelography are also worthy of mention, for they cause such severe pain that nephrectomy may be needed. We possess such cases, and many other authors before and since have drawn attention to similar cases.

The change in the bone marrow due the accumulation of Thorotrast can be compared with other ionizing actions: x-rays, radium, x-thorium, and atomic bombardment. The cases ascribed to Thorotrast were published by Spier and colleagues,[10] MacMahon and colleagues,[11] Birkner,[12] Schmidt and colleagues,[13] Rotter,[14] Lauda,[15] Moeschlin,[16] and Matthes.[17] We ourselves have two cases, not yet published, which appeared, respectively, 14 and 21 years after the injection of the substance.

The liver changes are produced very slowly and depend on the quantity of Thorotrast accumulated in the liver, the time elapsed since the injection, and the displacement of the substance in the organ. These changes give a picture which has been described by Albertini as "dystrophia lenta." Together with the more or less serious lesions of the liver cells, there appeared scar tissue at the sites of greatest accumulation of the substance—near the central veins, in the portal spaces, in the large biliary-portal spaces, and under Glisson's capsule. This displacement of Thorotrast toward the capsule is very slow, but as the years pass it becomes more and more important. It is in the very old cases, such as our material 15 to 25 years after injection, that the greatest deposits of the substance are found under the capsule and around them the longest scars. The external surface of the organ is depressed at the site of contact of these scars, giving a false coarse lobulation.

The oldest liver lesions produced by the Thorotrast have not been described with sufficient morphological detail. As far as we know, only Birkner and Rössle[18] referred to them in man with some detail. These authors speak of remodeling of the structure, accompanied by the formation of pseudolobules, and observed subcapsular scars. Rössle's opinion is that one cannot speak of a cirrhosis in the true sense of the word, as there exist many unaltered portal spaces. His expression, "an uncommon liver fibrosis," is worthy of being employed.

In three of our cases (autopsies done, respectively, 14, 22, and 25 years after the injection of Thorotrast) we found a clear pseudolobulation, but these pseudolobules were not enveloped by fibrous tissue. On the other hand, the pseudolobulation did not correspond to that seen on the external surface, which resulted, as we have said, only from retraction of the surface caused by the important subcapsular scars.

The liver changes, as referred to, are formed very slowly, and during a number of years the liver function tests are normal. In some of our oldest cases these tests were frankly positive. Other authors, such as Birkner, cite positive tests.

The work of Obiditsch and Obiditsch-Mayer[19] is of great interest. These authors describe one case, 10 years after arteriography, which showed, beside intensive positive liver function tests, curious alterations of blood proteins, such as reduced total proteins and increased globulins, especially the gamma fraction (electrophoresis). They explained this dysproteinemia by the liver changes and by the remarkable increase of plasmocytes formed in the bone marrow and in the liver. This dysproteinemia is thus partly due to the reaction of the reticuloendothelial system. Similar views were expressed by Rotter when, on presenting a case of panmyelopathy by Thorotrast, he mentioned findings which he interpreted as indicating a paraproteinosis: proliferation of the reticular cells of the bone marrow, with cytoplasmic inclusions of the "rod-shaped" type of Auer and the presence of a crystal in the product of the sternal puncture. The finding of amyloid substance in the spleen of a case reported by Lüdin[20] (death 14 years after the injection of Thorotrast for hemangioendothelioma of the liver and spleen) is also favorable to the same idea.

Finally, we would like to refer to the carcinogenic power of Thorotrast. This power of the ionizing radiations has long been known both through experimental work and through clinical cases. The latter are of great interest to us. Among them, we may mention the carcinomas due to the action of x-rays and radium (among others, Bauer §), the cases of osteosarcoma found among the female employees in the watch-making industry working with phosphorescent substances (Martland,[23] similar late cases reported by Aub and colleagues[24]), and the case reported by Gricouroff and colleagues of a sarcoma of the maxilla in a woman treated eight years before with injections of mesothorium bromide (Tavares ‖). The cases of carcinoma of the lung in the miners of Schneeberg and Joachimsthal have also been considered as caused by the action of radioactive dusts; however, as Brues[26] points out, the question is still open.

The carcinogenic action of the radioactive agents has been confirmed by numerous experiences. Carcinomas and sarcomas have been produced in animals of different kinds and at various sites in the body, such as the skin, the breast, the biliary ducts and liver, the kidney, the bones, and the meninges (Bauer).

Once these experimental and clinical facts became known, at the time when Thorotrast was beginning to be used as an x-ray contrast medium, some experimenters tried to obtain neoplasms with this substance.

The first tumors were produced in rats by Oberling and Guérin[27] (intraperitoneal injections; peritoneal sarcomatosis). The results were later confirmed by the same authors in collaboration with Roussy ¶ (subcutaneous and intraperitoneal injections). Selbie[30] produced fibromas, spindle-cell sarcomas, osteosarcomas, a histiocytoma, and a capillary angioendothelioma in mice. Foulds[31] obtained sarcomas and, for the first time, a carcinoma in guinea pigs by injections in the mammary region. Similar results were also obtained by Prussia, Myamotto, and Warren and associates. These authors are cited by Matthes.

The work of Onufrio,[32] van Mervennée and Ten Thiege, and Tavares and Morais, who obtained sarcomas in the internal organs by intravenous injections of Thorotrast, is of special interest in connection with the subject we are dealing with.

Recently Guimarães, Lamerton, and Christensen[33] obtained in white mice (albino mice) five hepatomas, one reticuloendothelioma of the liver, one hemangiothelioma of the spleen, and seven lung tumors also by using intravenous injections of the same substance. One of the hepatomas showed malignant characteristics. The lung tumors are of special interest; they were multiple and of an epithelial type, either solid or glandular and papillary. These tumors do not differ essentially from the spontaneous lung tumors and those produced experimentally by other means in albino mice.

Shortly after Thorotrast had been employed for the first time in man, its use was criticized because of what was known about the actions of the ionizing radiations, both in the clinical and in the experimental fields, and later, after 1933, because of the experimental production of tumors with this substance. The Council on Pharmacy and Chemistry of the American Medical Association[34] took a stand against the use of this substance after 1932. The Academie de Chirurgie, following a report by Béclère, Duval, Regaud, Rouhier, and Bazy (Béclère #) also condemned the use of Thorotrast, and the Swiss Society of Radiology did the same (Matthes).

In Germany the greatest efforts made toward abandoning this substance were due to K. H. Bauer. This author, comparing the time of appearance of neoplasms after the injections of Thorotrast, experimentally produced in animals, with their average life expectancy and the average span of life in

§ References 21, 22.

‖ Cited by Tavares and Morais.[28]

¶ References 28, 29.

Béclère, in discussion on Nicaud and Hamburger.[35]

man, came to the conclusion that such tumors would appear between the 12th and the 18th year after the injection in man.

Report of Case

Clinical Summary

A 46-year-old man, on Oct. 15, 1954, was taken with a dull, intermittent pain in the right hypochondrium, which radiated to the lumbar region on the same side and to the epigastric region. The pains were not related to the ingestion of food but became sharper with respiratory movements. He had an increased temperature (38 to 38.5 C [100.4 to 101.3 F.]). Four days later jaundice appeared. There was no nausea, vomiting, or loss of weight. When he was seen at the hospital, 15 days after the onset of the symptoms, the abdomen was protruding, but there was no collateral circulation and no ascites. Abdominal examination revealed an elastic, painless mass which seemed to be in continuity with the liver. The spleen was not palpable. A plain x-ray of the abdomen revealed an opacity of the spleen and liver, leading to the conclusion that the patient had been injected with Thorotrast. It was then found that the injection had been administered 22 years before. The purpose of the injection or how much drug had been given was not determinable. Three days before the patient's death, which occurred 20 days after the onset of the symptoms, the abdomen suddenly increased in volume, the pains became severer, and the general condition of the patient became progressively worse. On the day of death the patient was urgently transferred to a surgical department with the suspicion of an acute abdomen. He died, however, before surgical intervention was possible.

Autopsy.—The following are the most important data from the autopsy (Institute of Pathological Anatomy of Lisbon):

In the peritoneal cavity approximately 2 liters of liquid blood and clots was found.

Spleen: Very reduced in volume (4.5×3×1.5 cm.), with a pleated capsule. On the section, where no follicles were observed, shiny and sulfur-yellow punctuation and striae were seen.

Liver: Increased in volume (31×22×17 cm.). The capsule showed yellow striae and spots of irregular outline, which tended to conglomerate or formed a network. On the lower surface of the left lobe a break was found in Glisson's capsule, the size of the palm of a child's hand, through which a voluminous clot stood out. Section of the liver at this level showed that the external two-thirds of the lobe was replaced by a tumor mass of the angiomatous type (Fig. 1), formed by lacunar spaces full of liquid or coagulated blood. These spaces varied from the size of hemp grain to that of peeled almonds and were situated very close to one another, giving the whole structure a spongy aspect, similar to a honeycomb (Fig. 2). The existing septa between the blood cavities showed yellow and shiny striae. Between Glisson's capsule and the tumor mass, at the site at which the rupture was described, there was a large band of coagulated blood. The limit between the tumor and the liver parenchyma was not well defined, and for about 3 cm. a tran-

Fig. 1.—Aspect of the liver section. At the left the tumor; at the top an independent tumor formation.

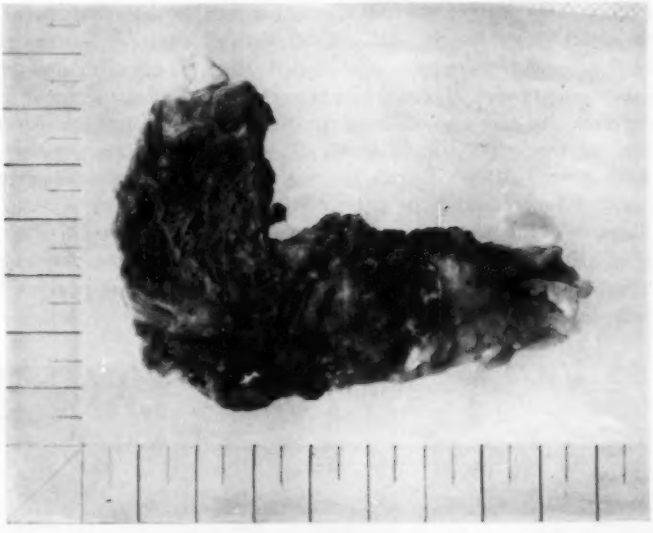

Fig. 2.—Section of the tumor. Vascular spaces visible.

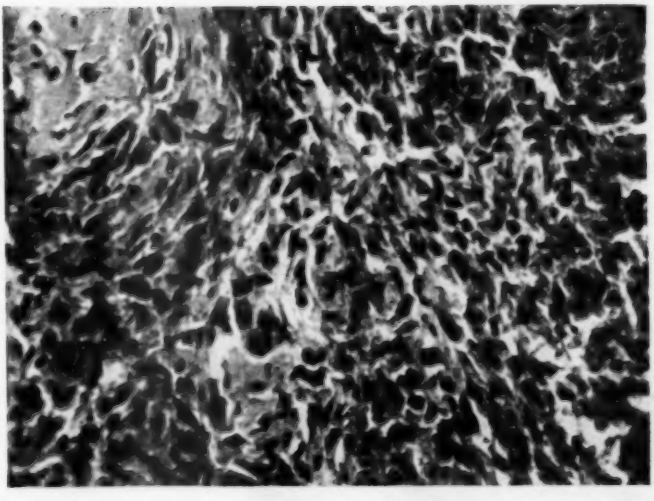

Fig. 3.—Very atypical solid areas.

sition could be seen. On the right lobe, various dark-red tumor formations, varying in size from corn grains to cherry seeds, were noted. These were scattered here and there and were sometimes formed by lacunar spaces filled with blood.

Macroscopic Diagnosis: Thorotrast in spleen, liver, and some of the lymph nodes of the liver hilus; angioblastic sarcoma of the left lobe of the liver; small tumor nodules in the right lobe; rupture of the tumor, with the formation of a subcapsular hematoma; hemoperitoneum and anemia.

Microscopic Examination

The tumor presented many aspects, consisting of blood lakes, small and irregular vascular clefts, reticular patterns, and, more rarely, solid areas. The last-mentioned portions (Fig. 3) were formed, as a rule, by small, oval, or elongated cells. These cells were almost limited to the nuclei, which were always rich in chromatin. This cellular type was the one most frequently met

with in any part of the neoplasm, but in the solid zones the atypical characteristics of the elements were more accentuated than in the other zones. There was a remarkable difference in size and an irregular disposition of the cells. Fibers, some of which stained with Van Gieson's method, were not uncommon among the cells; precollagen fibers were the most numerous. Even in the solid areas narrow clefts were seen. Reticular zones (Figs. 4 and 5), in which the cells anastomosed with one another at their tips, leaving spaces frequently full of erythrocytes between them, were numerous. These structures were mainly found where there was tumor proliferation between the liver trabeculae. The neoplastic elements may come together and circumscribe spaces which resemble capillaries or long and irregular clefts.

The walls (Fig. 6) of the cavities seen macroscopically and the others seen only on histological examination showed a variable structure. They were formed by

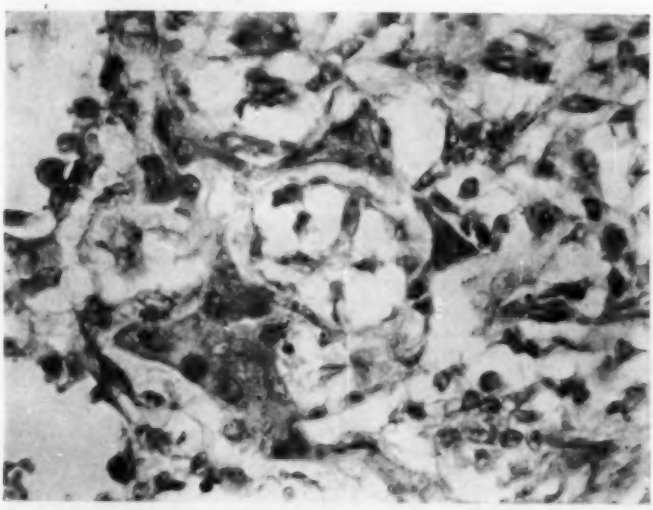

Fig. 4.—Areas of reticular proliferation.

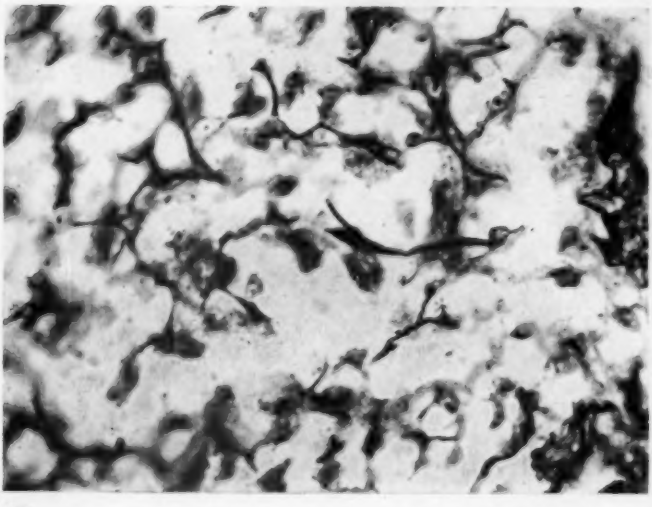

Fig. 5.—Silver-impregnated fibers in the reticular zones.

fibrous trabeculae, with or without tumor cells and with macrophages containing Thorotrast, by the tissue of the neoplasm itself, and even by liver trabeculae, whose intratrabecular spaces not uncommonly opened into the cavity. The content of these lacunae varied with the elements constituting the wall and consisted of fragments of tumors or isolated tumor cells, Thorotrast-laden macrophages, and liver cells. Rarely, there were numerous neutrophil granulocytes mixed with the above elements.

Normal liver structures, such as remains of hepatic-cell trabeculae, biliary-portal spaces containing abundant macrophages with Thorotrast, and scars with dense accumulations of this substance, were found in any section of the tumor examined.

The tumor invaded the biliary-portal spaces, where at times it formed clefts, on the edge of which the neoplastic cells were arranged like endothelial cells. One or another of the portal-vein ramifications were invaded by the tumor, as well as the lym-

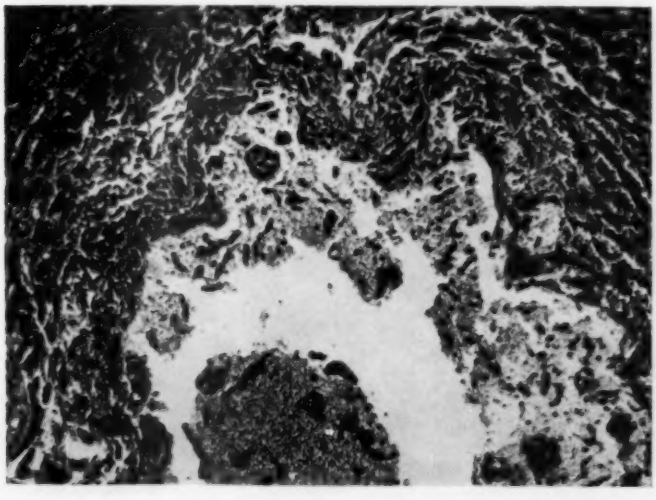

Fig. 6.—Bloody lacunae.

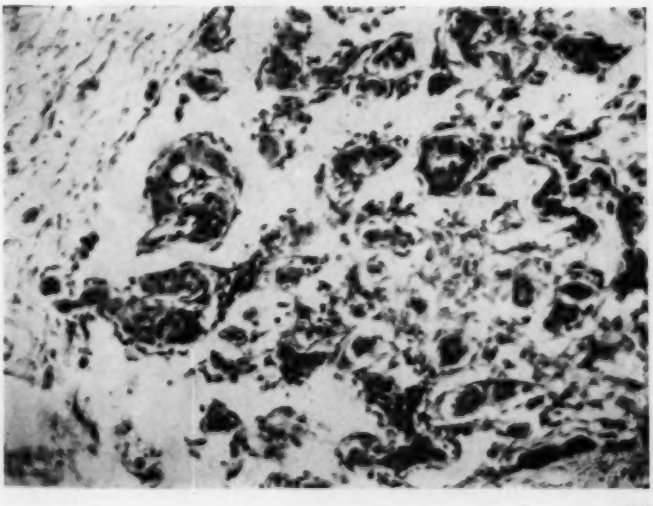

Fig. 7.—Proliferation of tumor cells on the edge of the hepatic trabeculae.

phatic channels, along which the cells of the tumor were also arranged as endothelial cells.

The disposition of the tumor cells between the liver trabeculae and on the edge of the trabeculae was of special interest, this pattern being mainly found at the zones where the liver was invaded (Figs. 7 to 12). At times the tumor cells were situated exactly at the site of the Kupffer cells, arranged in long rows along the trabeculae. In cross section the tumor cells were seen to be arranged like "sheaths" around a group of liver cells, or even around isolated cells. Between these "sheaths" and the liver cells it was not uncommon to find a space resembling a Disse space, in which erythrocytes (Fig. 11) and Thorotrast-laden phagocytes (Fig. 12) were seen. With stains for collagen and precollagen fibers, the tumor cells of the "sheaths" were seen to be situated on a thickened basal membrane, and the space existing between those and the liver cells was not uncommonly crossed by

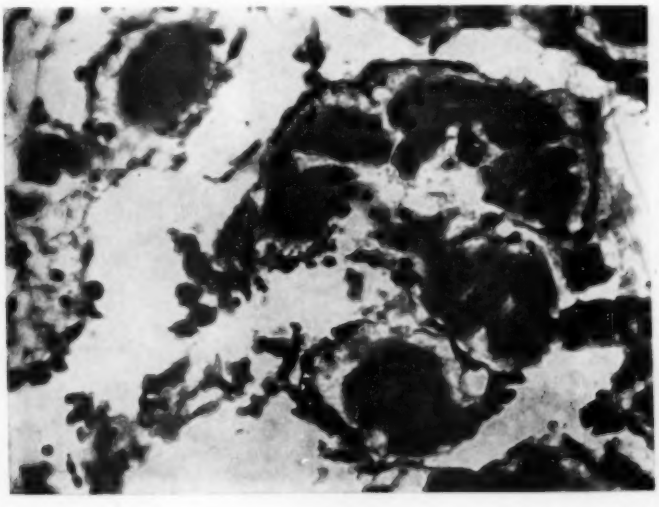

Fig. 8.—A group of liver cells surrounded by tumor sheaths.

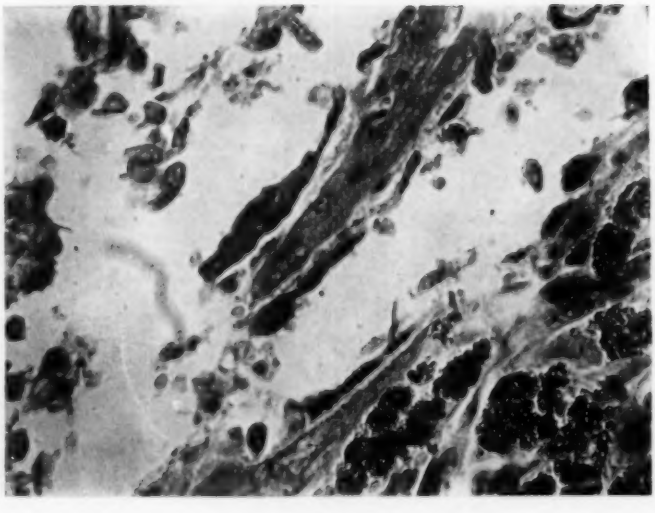

Fig. 9.—Tumor proliferation alongside a liver trabecula. Below, on the right, macrophages containing Thorotrast.

fibers (Fig. 13). The hepatic cells in these areas may be atrophied and show chromatolysis. Liver cells with their "sheaths" of tumor cells could be found loose in the blood of the lacunae (Figs. 6 and 10). The neoplastic elements that had proliferated between the liver trabeculae created several types of structures. Very often they formed a net of regular or irregular meshes. In the intermingled processes of the cells reticular fibers formed a small-meshed net (Fig. 5). There were also cellular proliferations forming a bridge from one trabecula to the other and solid structures which at times resembled a syncytium.

Large fibrous trabeculae containing Thorotrast crossed the most compact zones of the neoplasm. The tumor tissue in the independent nodules of the right lobe presented the above-mentioned lacunar type. Only very rarely did neoplastic cells contain Thorotrast.

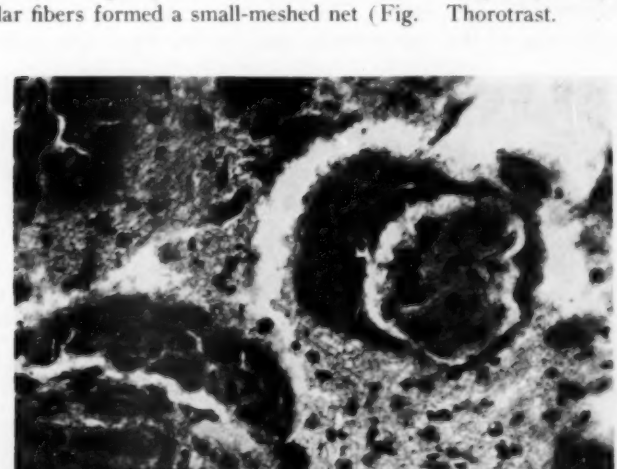

Fig. 10.—Hepatic trabeculae (arrows) surrounded by sheaths of tumor cells.

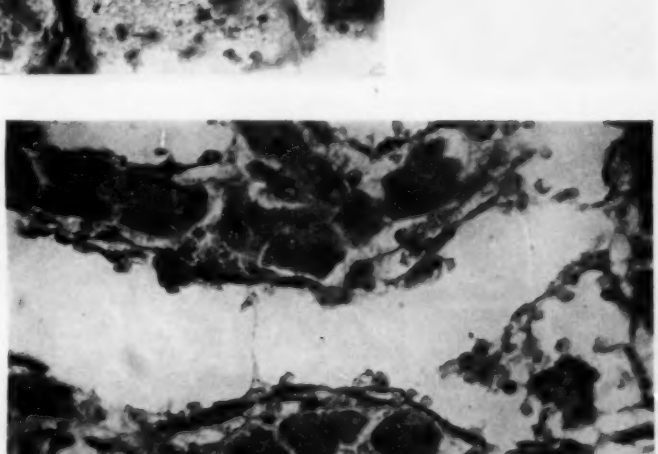

Fig. 11. — Between tumor cells and liver cells a space full of erythrocytes.

Comment

The tumor we have described has sufficient histological characteristics to be considered malignant, especially in the solid zones.

In summary, we observed a sarcoma of the liver appearing in a man who 22 years before had had injections of Thorotrast, most likely for aortography. The quantity injected could not be determined. The tumor was situated in the left lobe of the liver. In the right lobe there were independent tumor nodules. Death resulted, as in the case reported by MacMahon, from hemoperitoneum following rupture of the tumor. This tumor, considering the structure presented, is sure to have originated from the endothelial cells lining the sinusoids of the liver lobule. We consider the origin of the tumor proliferation as occurring between the trabeculae and around them. The tumor elements showed reticular potentialities and formed blood spaces, be-

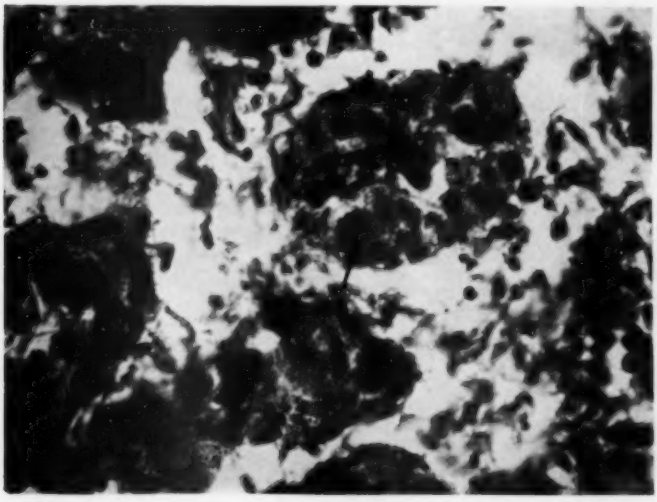

Fig. 12.—Macrophages containing Thorotrast in the space shown in Figure 11. Lower arrow, macrophages; upper arrow, liver cells.

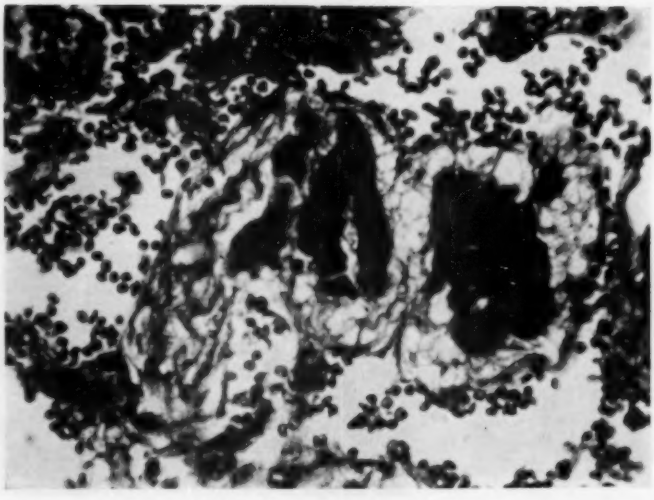

Fig. 13.—Impregnation by silver. Proliferation of reticular fibers, which form a web in the space between the tumor cells and the hepatic trabeculae.

having frequently like endothelial cells.

As can be gathered from the study of radiations made by Prof. O. Eichler (Table 1), and even from the histological examination of our sections, much Thorotrast is to be found in the tumor, but the latter examination shows that the Thorotrast-laden phagocytes are situated in great quantities in the scars and in the great biliary-portal spaces, as well as between the "sheaths" of tumor cells and the liver trabeculae. Are these Thorotrast-laden phagocytes tumor cells? We believe that the tumor was formed in a zone where the denser deposits of Thorotrast were situated. Our experience with the examination of old and very old cases permits us to state that the drug accumulates most densely in the subcapsular areas. It is natural for the tumor to have originated in these areas, and the situation of the tumor in the case presented supports this assumption.

Owing to the independence of the venous circulation of the right and the left lobe, we think that the several tumors of the right lobe may not be metastatic but that we may be in presence of a tumor of multicentric origin.

An important characteristic of the tumor is the role which the cells seem to play in the formation of the fibers. In fact, the neoplasm, besides many reticular and collagen fibers—the latter in the solid zones—presents special aspects which lead us to this conclusion. As soon as tumor cells appear along the trabeculae, the *Gitterfasern* are thickened and form a kind of wide basal membrane which can be stained by Mallory's method, impregnated with silver, and also stained red by Van Gieson's method. On the other hand, Figure 13 shows that the space formed between the liver cells and the tumor edge is crossed by numerous fibers, which constitute a kind of a web in this zone. Later a large band of collagen can be seen between the tumor edge and the atrophied liver cells.

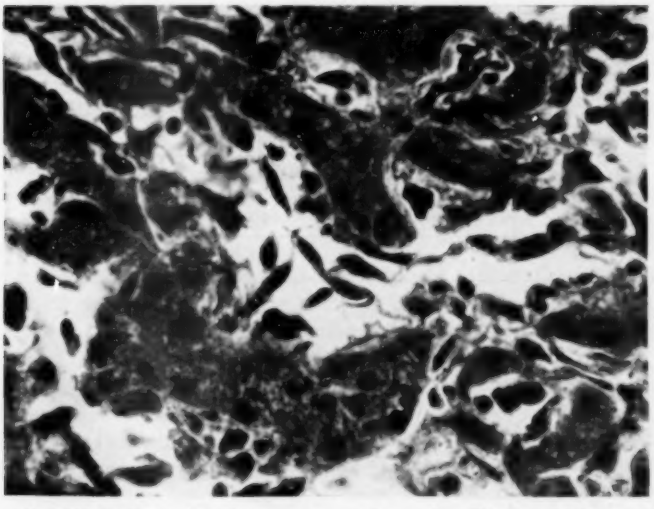

Fig. 14.—Proliferation of tumor cells between the liver trabeculae.

Finally, the liver trabeculae have disappeared, and in their place a thick, fibrous septum remains.

We find 15 cases of tumors (Table 2) considered by various authors as resulting from the action of thorium dioxide. Not all of them can be accepted without criticism. As regards carcinoma, the most important reason for accepting the assumption that a certain case had such an origin is that the tumor is formed in the neighborhood of an important deposit of Thorotrast. Such is the case presented by Hofer[38] (squamous-cell carcinoma), in which the substance was retained in a maxillary sinus for 10 years.

TABLE 1.—*Determinations of Radioactivity*

	Contents of Ashes	Impulses per Mg. Ashes/Min.	Impulses per Gram Weight of Fresh Material/Min.	Real β Disintegration/Min.
Liver*	0.89	2.09	26.6	364.0
Liver	2.82	6.10	172.0	2,356.0
Liver	3.73	7.01	261.3	3,580.0
Tumor	0.784	4.73	37.1	508.4
Spleen	4.30	7.52	33.0	451.5

	β Disintegration/Min. Inside Tissue	α Disintegration/Min.	α+β Disintegration/Min.	Percentages of Thorotrast in Ashes
Liver	1,456	2,200	3,656	17.2
Liver	9,424	14,150	23,574	35.1
Liver	14,320	21,500	35,820	40.3
Tumor	2,025.6	3,040	5,065.6	27.2
Spleen	1,806.0	2,710.0	4,516.0	43.2

* Several fragments.
Prof. Dr. O. Eichler, Heidelberg, Germany, made the above determinations.

TABLE 2.—*Cases of Tumors Considered to be Due to Thorotrast*

	Year	Purpose of Injection	Latent Period, Yr.	Tumor
MacMahon	1947	Hepatosplenography	12	Endothelial sarcoma, liver
Zollinger	1949	Pyelography	16	Spindle-cell sarcoma, kidney
Rudolphi	1950	Dacryocystography	35	Squamous-cell carcinoma
Abrahamson et al.	1951	Hepatosplenography	16	Multicentric alveolar-cell carcinoma, lung
Silva Horta (first case)	1951	Cerebral angiography	31/6	Endothelial sarcoma, liver
Hofer	1952	Maxillary visualization	10	Squamous-cell carcinoma
Vögtlin and Minder	1952	Bronchography	18	Squamous-cell carcinoma, bronchus
Lödin	1953	Arteriography	14	Hemangioendothelioma, liver and spleen
Matthes	1954	Hepatosplenography	21	Malignant hepatoma
Hettmann	1954	Hepatosplenography	20	Carcinoma of bile duct
Plenge	1954	Cerebral angiography	6	Sarcoma at site of injection
Tesluk and Nordin	1955	Cerebral angiography	14	Hemangioendothelioma of liver
Fruhling and others	1955	Arteriography	12	Malignant hemangioendothelioma of liver, spleen, and bone marrow
Grossiord and others	1956	Arteriography	21	Adenocarcinoma
Silva Horta (second case)	1956	Probably aortography	22	Malignant hemangioendothelioma of liver

The same can be said of the case reported by Rudolphi,[37] and probably of that of Matthes [17] (malignant hepatoma). On the other hand, we think that the case of Abrahamson, O'Connor, and Abrahamson [38] of multicentric alveolar carcinoma of the lung is to be rejected. The authors themselves confess that they could find no Thorotrast in the lung. In Vögtlin and Minder's [39] case (squamous-cell carcinoma of the lung—bronchography with Thorotrast 18 years before) there was a considerable quantity of the drug in the lung.

Recently (1956) Hackenthal [40] described a case of a carcinoma of the left main bronchus (anaplastic carcinoma) in a man who 16 years before had had injection of Thorotrast. This author does not hold the aforesaid drug to be responsible for the appearance of the tumor, although he found some accumulations of Thorotrast in the proximity of the neoplasm. In this instance, however, the very important objection regarding the present high frequency of carcinoma of bronchus must be considered. The role of the radiation as a cause of the tumor cannot, therefore, be affirmed or denied. Heitmann's [41] case (carcinoma of the bile duct) in a patient in whom Thorotrast was injected intravenously 20 years before (hepatosplenography) should be rejected. The tumor did not develop near deposits of the drug. The author himself refutes this etiology; Brunner [42] and Tesluk and Nordin [43] came to the same conclusion.

In the case of Grossiord,[44] 21 years after arteriography, there was cirrhosis and a cholangioma with metastasis to the liver. The author submits that the tumor originated in the pseudotubules, pointing out transitional aspects between these structures and the neoplasm. Although no references are made to histological details with regard to the type of cirrhosis found, we believe, nevertheless, taking into account the description of the case, that any ordinary type of cirrhosis should be excluded and, rather, that deposits of thorium dioxide should be held responsible for the cirrhosis.

It will be interesting to check in the future whether tumors of the kind will be found in connection with cirrhosis produced by Thorotrast. We believe, in this connection, that an aspect we found in a case of cirrhosis from Thorotrast is of particular importance. This consisted of an adenomatous node resulting from the proliferation of pseudotubules, which can be seen by the naked eye in the histological section.

As to the mesodermal tumors in which Thorotrast has been considered the cause, we must point out Plenge and Krückemeyer's [45] case (fibrosarcoma) as being, in our opinion, the only tumor formed, up to date, in the tissues surrounding the vessels into which the drug was injected, i. e., in the place of the "paravascular granulomas." We have examined many Thorotrast granulomas and have never found the cellular atypias which can lead to the diagnosis of sarcoma. In Zollinger's [46] case (spindle-cell sarcoma) the tumor was related to important deposits of the drug due to an ascending pyelography. This case does not seem to be criticizable.

The mesodermal tumors which have not yet been discussed are located in an organ which accumulates much Thorotrast—the liver—one of them is situated also in another great accumulator of Thorotrast—the spleen (Lüdin). The article of Fruhling [47] arouses special interest with regard to localization of the tumors in the three reticuloendothelial organs—spleen, liver, and bone marrow.

The tumors present a close histological relationship, and it can be stated that the type of neoplasm is the same—a hemangioendothelioma. From case to case the only differences are in the secondary structures and in the presence or absence of atypical cytological characteristics, upon which the diagnosis of sarcoma was based in four of six cases.

There is even a certain parallelism in the evolution of these cases, as Tesluk and Nordin pointed out existed between their case and that of MacMahon. The hemorrhagic characteristics of the lesions existed in both cases, in Lüdin's and in our second

case, which had as a terminal feature a hemoperitoneum caused by the rupture of the tumor, such as occurred in MacMahon's case. The histological aspects, as we have pointed out, are similar, as shown, for instance, by our second case and by those of Lüdin and Tesluk. The similarity, for instance, between Figure 4 of the article by the latter authors and our Figure 7 is remarkable.

With regard to tumors of organs that accumulate Thorotrast, we think that the structural identity of the cases is a strong reason to consider them as being due to the radiations of thorium dioxide. In connection with this, we must keep in mind that Zeitlhofer and Speiser [48] obtained by means of intravenous injections of the substance in the rabbit a hemangioendothelioma of the spleen and liver, which caused death by hemoperitoneum due to rupture of one of the tumors of the spleen. These authors, on their part, call attention to the similarity of the structures they found to those of Lüdin's case. We do not think that in the presence of such facts the objections put to MacMahon's case have much value.

Only our first case remains to be discussed. The structures of the tumor are like the others; however, as we pointed out in 1951 and 1953, we have to consider the time factor, as there is a latent period only of three years two months. Bauer thinks that this case should be considered with those that have been ascribed to the action of Thorotrast (Silva Horta).

Other factors eventually interfering in the determination of the moment of appearance of the neoplasm will perhaps justify such a short latent period.

In short, according to our opinion, we consider as definite cases those of MacMahon, Zollinger, Rudolphi, Lüdin, Hofer, Tesluk and Nordin, Fruhling, and Silva Horta (second case); as probable cases, those of Plenge, Matthes, and Silva Horta (first case), and Grossiord; as doubtful cases, that of Vögtlin and Grossiord, and as cases to be excluded, those of Abrahamson and Heitmann.

Certain morphological pictures appearing in patients given injections of Thorotrast are of interest, as they may represent stages of transition to neoplasm produced by this substance. Various authors have called attention to this point, and Lüdin refers to the fact that Sheideger found certain atypical characteristics in the proliferated cells of the splenic pulp in the neighborhood of the deposits of this substance in the spleen six years after an encephalography. The nodular proliferations of Kupffer cells verified by Rotter in a fragment of liver obtained at biopsy (Thorotrast injection 12 years before) points to the same conclusion. This author calls attention to the importance that such pictures can have in the genesis of malignant tumors. The bone marrow itself, in the cases of panmyelopathy due to Thorotrast, presents a hyperplastic aspect which resembles very much neoplastic proliferation. This is what happened in the cases of Rotter and of Schmidt and associates. In the latter case the authors state that the picture found would justify the term myelomatosis. The diffuse proliferation of Kupffer cells verified in the case of Groskopff, Bolck, and Bühl must have, according to Lüdin, the same significance.

Summary

The morbid aspects caused by the introduction of Thorotrast in the human organism are noted, and a new case of angioblastic sarcoma of the liver which appeared in a man who 22 years before had received injections of the substance in question, is presented. Death resulted from hemoperitoneum.

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Studies of the Adrenal Glands of Patients with Low Plasma Sodium

JOHN NICHOLS, M.D., New Haven, Conn.

Considerable evidence has accumulated indicating that the inner zones of the adrenal cortex are under the influence of the pituitary gland and are concerned with formation of the cortisone type of hormone, whereas the outer, the zona glomerulosa, is independent of the pituitary and is concerned mainly with the elaboration of the sodium-retaining type of hormone (aldosterone). These conclusions are derived chiefly from the fact that the zona glomerulosa does not undergo atrophy after hypophysectomy and that the hypophysectomized animal does not show gross impairment of electrolyte balance. In the experimental animal the feeding of diet high or low in sodium or potassium causes histological changes in the zona glomerulosa, thereby suggesting that the functional activity of this zone has an inverse relation to the plasma level of these ions. All of the evidence derived from electrolyte levels has been in experimental animals, except for the single report of Peschel and Race,[1] who on examination of sections with hematoxylin and eosin found hypertrophy of the zona glomerulosa of patients who died with low plasma sodium values. Because the cortical hormones are probably formed from cholesterol, and a decrease in the cholesterol content after stimulation is usually taken as evidence of hormone formation, it was deemed advisable to make a histological study of the cholesterol distribution in the adrenals of patients who died after gross electrolyte impairment. These findings are compared with those in cases of shock from prolonged trauma and in cases of sudden death of otherwise healthy persons.

Submitted for publication May 17, 1956.

Presented at the 20th International Physiological Congress, Brussels, July 30, 1956.

Department of Pathology, Yale University School of Medicine. Present address: Department of Pathology, Medical College of Virginia, Richmond, Va.

Materials and Methods

For this study the adrenals of all patients for the previous five years who had not received hormone therapy and who had maintained a plasma sodium value of less than 120 mEq/L for more than a week prior to death were recovered from the formalin storage bottles. Hematoxylin and eosin preparations were remade, and the other portions of the glands were frozen, sectioned, and stained with Sudan III for total lipids and with the Schultz reagent for total cholesterol. From the rather large number of cases, most of which had complicating factors which confused the interpretation, 11 were selected for this report. These were of patients treated with a low-sodium diet for hypertension and who had almost identical clinical courses and the findings on whom were unequivocal. The adrenals for comparison were from patients dying of multiple fractures following trauma, such as automobile accidents, and who survived more than three days, with normal plasma sodium values. The "normal" adrenals were obtained from cases of sudden death, such as acute and sudden trauma of automobile accidents and coronary thrombosis.

Results

Figure 1 shows the distribution of total lipids as revealed by the Sudan III stain in a "normal" gland. As can be seen, the zona glomerulosa stains prominently and is well demarcated from the zona fasciculata and zona reticularis, which stain less intensely as they approach the medulla. Figure 2 shows the distribution of cholesterol as revealed by the Schultz reaction in the same case; here it can be seen that the cholesterol

Fig. 1.—Normal human adrenal cortex stained for total lipids with Sudan III. Note normal distribution of lipids.

Fig. 2.—Normal human adrenal cortex from another case than that in Figure 1, stained with the Schultz reagent for total cholesterol. Note normal distribution of cholesterol.

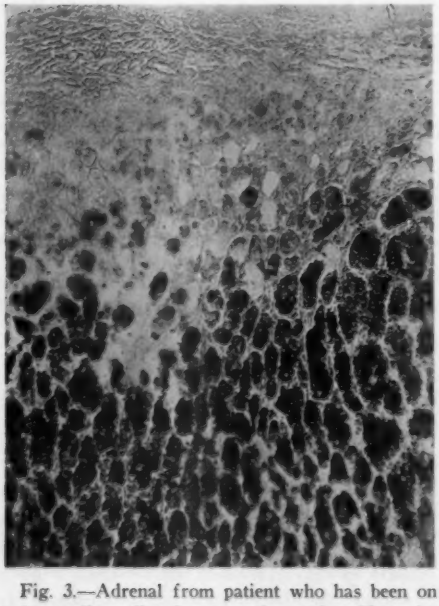

Fig. 3.—Adrenal from patient who has been on a low-sodium diet for several months. Note depletion of total lipids from zona glomerulosa, as revealed by Sudan stain.

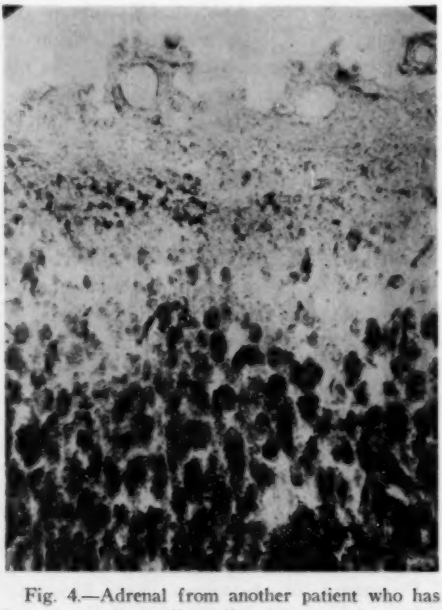

Fig. 4.—Adrenal from another patient who has been on a low-sodium diet for several months. Note depletion of cholesterol from zona glomerulosa, as revealed by Schultz reagent.

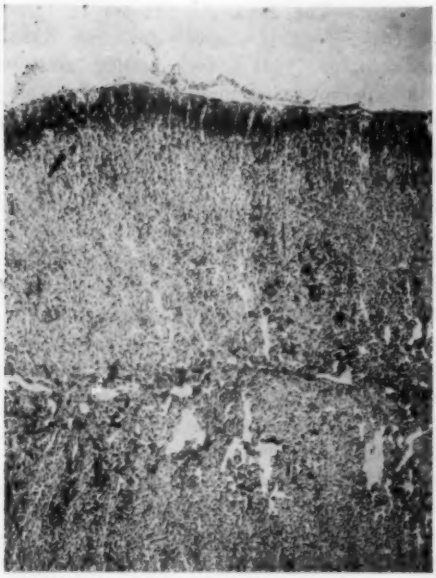

Fig. 5.—Adrenal from patient dying of shock from multiple fractures on the fifth day. Note depletion of lipids from inner zones, as revealed by Sudan stain. The zona glomerulosa is relatively normal.

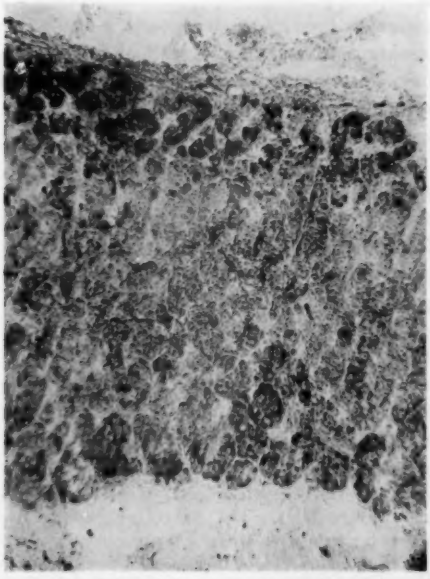

Fig. 6.—Adrenal from another patient dying of traumatic shock, stained with the Schultz reagent. Note cholesterol is relatively well preserved in the zona glomerulosa, while it is depleted in the inner zones.

is distributed almost equally throughout the zones, but with lessened intensity as the medulla is approached.

Figure 3 shows the distribution of lipids in a patient who was on a low-sodium diet for several months for hypertension and who for the previous two weeks had a plasma sodium level ranging from 110 to 120 mEq/L. This patient had ascites and marked generalized edema. Digitalis and mercurial diuretics had been given, but no saline or hormone therapy had been instituted. Here it can be seen that the zona glomerulosa has been depleted of lipids to a much greater degree than has the two inner zones, presumably in an attempt to form the sodium-retaining aldosterone (electrocortin). Figure 4 shows the cholesterol, which comprises approximately 60% of the lipids of the gland, to be depleted in a pattern similar to that of the total lipids. Under these circumstances, it is reasonable to conclude that the cholesterol has been utilized in the formation of hormone.

Figure 5 shows the distribution of total lipids in a patient who suffered multiple fractures of the chest and pelvis and who survived five days. Plasma sodium determinations done on the third day after injury and a few hours before death revealed a level of 135 and 131 mEq/L respectively. It can be seen that the lipids have become depleted to the greatest extent in the inner zones, whereas the zona glomerulosa is relatively unchanged. This depletion of lipids in the inner zones is presumably due to the formation of hormones of the nonelectrolyte type under stimulation of corticotropin. Two eosinophile counts on this patient, on the fourth and fifth days, were 40 and zero respectively. In Figure 6 it can be seen that the cholesterol distribution is almost identical with that of the total lipids.

Sudan and Schultz stains on the adrenals from the other 10 cases of uncomplicated low sodium values, ranging from 110 to 120 mEq/L for two to four weeks prior to death revealed almost identical findings with those shown in Figures 3 and 4. A series of

adrenals from cases of sudden death were identical with those in Figures 1 and 2. A series of adrenals from patients dying after prolonged trauma showed depletion of cholesterol from the inner zones almost identical with that shown in Figures 5 and 6. This depletion of lipids in the inner zones is found in most patients who have been in shock prior to death and is identical with that in the adrenal after corticotropin administration.[2] Sarason[3] and Symington and associates[4] have described the adrenal under various conditions of stress and have adequately documented the various patterns of lipid depletion apart from the stress of low sodium.

Comment

The function of cholesterol in the adrenal and its intensity as revealed by the Schultz reaction are far from settled, but there is enough indirect evidence, such as that reported here, to suggest that depletion of cholesterol in a stressful situation means glandular activity. Sayers,[5] in his review, states that the galaxy of histochemical tests for steroids has added little beyond what is obtained by simple Sudan stains.

The finding of cholesterol depletion in the zona glomerulosa of the adrenals of patients with low plasma sodium is in keeping with the observations of Peschel and Race,[1] who observed hypertrophy of the zona glomerulosa in patients dying during the course of therapy of a low-sodium rice diet for hypertension. Indeed, inspection of the adrenals of these patients with hematoxylin and eosin stains shows the zona glomerulosa is probably hypertrophied. However, it is difficult to differentiate the boundary between the zona glomerulosa and zona fasciculata.

The most conclusive evidence that the zona glomerulosa regulates electrolyte metabolism is the fact that the patient with hypopituitarism (Simmonds' disease) or the patient with postpartum necrosis of the pituitary (Sheehan's syndrome) shows no gross impairment of sodium balance, whereas the other functions of the adrenal are deficient. The zona glomerulosa in these patients is intact, whereas the inner zones are atrophic. This is the situation, also, in the experimental animal. Further, the administration of the synthetic sodium-retaining desoxycorticosterone causes atrophy of the zona glomerulosa and depletion of cholesterol in the rat's adrenal.[7] This depletion of cholesterol from the zona glomerulosa in the rat can also be obtained by force feeding a high-sodium diet[8] and is interpreted as a disuse phenomenon. On the other hand, the zona glomerulosa of the rat's adrenal can be depleted of cholesterol by feeding a low-sodium diet,[9] and, further, this can be obtained in the hypophysectomized rat,[10] indicating that corticotropin is not necessary. The stress of the low-sodium diets in the rats is interpreted as accentuating the need for an excess amount of sodium-retaining compound. It is probable that the zona glomerulosa in the dog also regulates electrolyte metabolism because dogs with intact zona glomerulosa and atrophied zona fasciculata and reticularis from DDD (2, 2-bis-[*P*-chlorophenyl]-1, 1-dichloroethane) administration[11] can withstand a crude potassium tolerance test. Race * and associates have recently reported hypertrophy of the zona glomerulosa in dogs maintained on a low-sodium diet.

Further, dogs given prolonged cortisone treatment develop low levels of circulating ACTH with resultant atrophy of the zona fasciculata and zona reticularis. These dogs have normal zona glomerulosa and excrete normal amounts of aldosterone.[12]

Patients with completely atrophic adrenals in Addison's disease are in electrolyte imbalance and do not secrete aldosterone in their urine, whereas patients with an intact zona glomerulosa, in cases of hypopitui-

* Race, G. W.; Nickey, W. A.; Wolf, P. S., and Jordan, E. J.: The Effects of Sodium Restriction and Potassium Excess on the Dog Adrenal Gland: A Possible Relation Between Morphologic Changes and Serum Electrolyte Concentration, read at the 53d Annual Meeting of the American Association of Pathologists and Bacteriologists, Cincinnati, April 26-28, 1956.

tarism, are in electrolyte balance and secrete normal amounts of aldosterone.[18]

The most convincing evidence that the zona glomerulosa secretes aldosterone is the finding by Ayers[14] and Girout[15] and their associates that slices of the outer zones of the cortex secrete more aldosterone in vitro during incubation than do the inner zones.

Chester Jones and associates † presented experiments in which the rat with an enucleated adrenal was allowed to drink either tap water or saline ad lib. The regenerating zones had the morphological appearance of the zona fasciculata. Further, these authors concluded that the zona glomerulosa is an inactive zone, to be called into use whenever an appropriate stress is applied. These workers also reported that when the adrenal cortex is hyperactive there is a diminution in the size of the zona glomerulosa, so that in markedly active adrenals the zona glomerulosa disappears altogether—the zona fasciculata then extends to the outer connective-tissue capsule. This architecture can be seen in (*a*) the hyperactive adrenals of the rat with diabetes insipidus and (*b*) after the administration of corticotropin to the normal rat.

The idea that the zona glomerulosa regulates electrolyte metabolism is further complicated by the fact that in a case of primary aldosteronism from a unilateral tumor, the tumor had the appearance of arising from the zona fasciculata. On examination of the contralateral adrenal, atrophy was seen in the zona fasciculata.[18] It would seem that under neoplastic conditions and under some experimental conditions the physiological mechanisms do not apply.

Summary

The adrenal glands of 11 uncomplicated cases of low plasma sodium from patients treated for hypertension with a low-sodium diet showed depletion of cholesterol in the zona glomerulosa. This is contrasted with the depletion of the inner zones in cases of traumatic shock and is interpreted as further evidence that the outer zones of the adrenal glands secrete the electrolyte-regulating hormone(s).

† References 16, 17.

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News and Comment

PERSONAL

Dr. Edgar M. Medlar Dies.—Dr. Edgar M. Medlar, principal pathologist for the New York State Tuberculosis Service at Hermann M. Biggs Memorial Hospital, Ithaca, and well known for his contributions to the pathology of tuberculosis, died in Ithaca on June 30, 1956, at the age of 69.

Appointment for Dr. David G. Freiman.—Dr. David G. Freiman has been appointed clinical professor of pathology at the Harvard Medical School. Dr. Freiman is also pathologist-in-chief at the Beth Israel Hospital, Boston.

Appointment for Dr. Edith L. Potter.—Dr. Edith L. Potter has been made Professor of Pathology in the Department of Obstetrics and Gynecology at the University of Chicago School of Medicine.

DEATHS

Dr. Harry R. Wahl.—Dr. Harry R. Wahl, Professor of Pathology at the University of Kansas Medical Center, died on June 18, 1956. Dr. Wahl was Chairman of the Department of Pathology at the University of Kansas from 1919 until 1951 and was to have retired on July 1, 1956. He also served as Acting Dean of the University of Kansas School of Medicine from 1924 until 1927 and was Dean from 1927 to 1948. Early in the spring of 1956, one month before his death, the Board of Regents at the University of Kansas voted unanimously that the present Medical Sciences Building be renamed Wahl Hall in honor of Dr. Wahl's many years of service to the University.

ANNOUNCEMENTS

Refresher Laboratory Training Courses.—Refresher laboratory training courses are being offered by the U. S. Department of Health, Education and Welfare, Public Health Service, as follows.

Title: Laboratory Methods in the Diagnosis of Bacterial Diseases: Part II. General Bacteriology.
Place and Dates Course Offered: Chamblee, Ga., Sept. 24-Oct. 5, 1956.
Fees: None.

Title: Laboratory Methods in the Diagnosis of Bacterial Diseases, Enteric Bacteriology.
Place and Dates Course Offered: Chamblee, Ga., Oct. 8-19, 1956.
Fees: None.

Title: Laboratory Methods in the Diagnosis of Viral and Rickettsial Diseases.
Place and Dates Course Offered: Montgomery, Ala., Oct. 15-26, 1956.
Fees: None.

Title: Laboratory Methods in the Diagnosis of Parasitic Diseases: Part II. Blood Parasites.
Place and Dates Offered: Chamblee, Ga., Oct. 8-26, 1956.
Fees: None.

Title: Laboratory Methods in the Diagnosis of Rabies.
Place and Dates Course Offered: Montgomery, Ala., Oct. 29-Nov. 2, 1956.
Fees: None.

Title: Laboratory Methods in Medical Mycology: Part I. Cutaneous Pathogenic Fungi.
Place and Dates Course Offered: Chamblee, Ga., Jan. 7-18, 1957.
Fees: None.

Title: Laboratory Methods in Medical Mycology: Part II. Subcutaneous and Systemic Fungi.
Place and Dates Course Offered: Chamblee, Ga., Jan. 21-Feb. 1, 1957.
Fees: None.

Title: Laboratory Methods in the Diagnosis of Tuberculosis.
Place and Dates Course Offered: Chamblee, Ga., Jan. 21-Feb. 1, 1957.
Fees: None.

Title: Laboratory Methods in the Study of Pulmonary Mycoses.
Place and Dates Course Offered: Chamblee, Ga., Feb. 4-15, 1957.

Fees: None.
Title: Laboratory Diagnostic Methods in Veterinary Mycology.
Place and Dates Course Offered: Chamblee, Ga., Feb. 25-March 1, 1957.
Fees: None.
Title: Laboratory Methods in the Diagnosis of Viral and Rickettsial Diseases.
Place and Dates Course Offered: Montgomery, Ala., March 11-22, 1957.
Fees: None.
Title: Serologic Methods in the Diagnosis of Parasitic and Mycotic Infections.
Place and Dates Course Offered: Chamblee, Ga., March 11-22, 1957.
Fees: None.

Application forms or additional information concerning these courses may be obtained from Laboratory Training Services, Communicable Disease Center, P. O. Box 185, Chamblee, Ga., or from the state health officer.

Books

Progress in Hematology. Vol. I. Edited by Leandro M. Tocantins, with 27 contributors. Price, $9.75. Pp. 336. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1956.

This very nice review monograph compiles information on a wide range of topics in modern hematologic investigations. Its contents include such subjects as gastric intrinsic factor and cyanocobalamin (vitamin B_{12}); parenterally administered iron in treatment of hypochromic anemia; life span of erythrocytes and their post-transfusion survival; abnormal hemoglobins; systemic lupus erythematosus and the blood; the autoimmune thrombocytopenias, and biochemistry and enzymatic activities of leucocytes in health and disease. In all, there are 16 articles, many by outstanding investigators in the particular areas. Figures, charts, and pictures are well reproduced; they are for the most part clearly presented. All interested in the modern chemistry, immunology, and biology of the blood and blood-forming tissues will find useful material in this volume; it will be of most service to those who wish to survey the areas included.

Cytology of the Blood and Blood-Forming Organs. By Marcel Bessis; translated from the French by Eric Ponder. Price, $22.00. Pp. 629, with 405 figures. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1956.

Modern microscopic morphology turns more and more to the use of histochemical and histophysical data, including the higher resolutions of the electron microscope. This volume is primarily a treatise on cellular morphology. It differs from any book thus far appearing on the American scene in containing a substantial quantity of electron microscopic studies, studies with polarization optics and phase contrast of various blood cell types in both health and disease. The photographic reproductions are most pleasing and sharp in their detail. For this alone the book would be interesting to pathologists. In addition to this there is a large amount of technical recipe, well described, and a good deal of excellent biology and pathology. For anyone interested in hematology this volume provides material compiled in one place which can be gathered only with difficulty from the literature.

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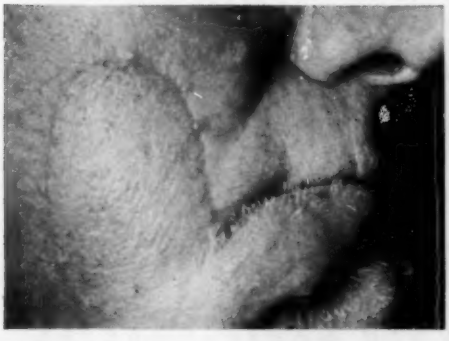

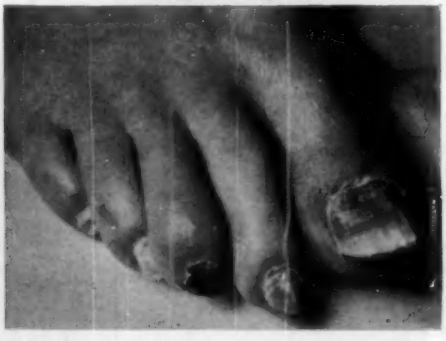

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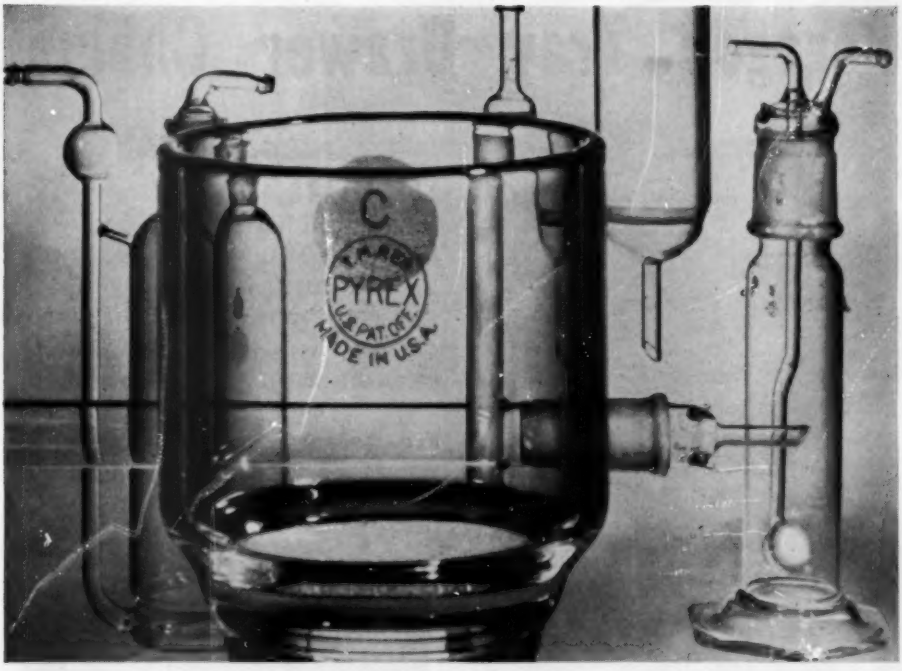

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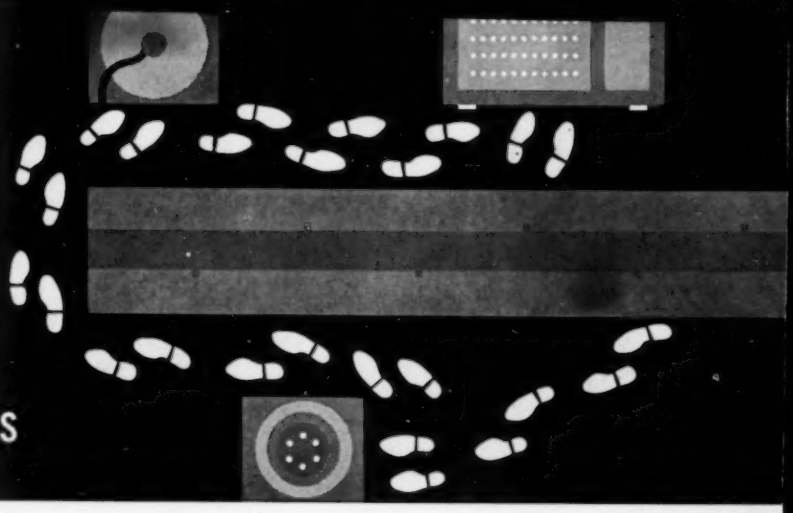

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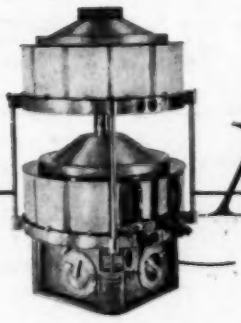

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